DEVELOPEMENT AND VALIDATION OF DAPOXETINE IN PURE AND SOLID DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRIC METHODS AND HPTLC METHOD

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MASTER OF PHARMACY

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Submitted by

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MELMARUVATHUR - 603 319

MAY 2012

CERTIFICATE

This is to certify that the research work entitled **"DEVELOPEMENT AND VALIDATION OF DAPOXETINE IN PURE AND SOLID DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRIC METHODS AND HPTLC"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **R.VELMURUGAN (Register No. 26106131)** in the Department of Pharmaceutical Analysis under our direct guidance and supervision during the academic year 2011-2012.

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DEDICATED TO
MY
PARENTS, SISTERS
AND TO
MY LOVED ONES

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DEDICATED TO
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CONTENTS

LIST OF FIGURES

13.	LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD (2 µg/mL)
14.	LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD (3 µg/mL)
15.	LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD (4 µg/mL)
16.	LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD (5 µg/mL)
17.	LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD (6 µg/mL)
18.	CALIBRATION CURVE OF DAPOXETINE BY HPTLC METHOD AT 437 nm
19.	CHROMATOGRAM OF ANALYSIS OF FORMULATION (PRILIYXET 30)
20.	CHROMATOGRAM OF RECOVERY OF FORMULATION (PRILIYXET 30)

LIST OF TABLES

14.	RECOVERY STUDIES FOR FORMULATION (PRILYXET 30) BY DERIVATIVE METHOD
15.	INTERDAY AND INTRADAY ANALYSIS OF FORMULATION (PRILYXET 30) BY DERIVATIVE METHOD
16.	RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY DERIVATIVE METHOD
17.	OPTICAL CHARACTERISTICS DAPOXETINE OF BY VISIBLE SPECTROPHOTOMETRIC METHOD
18.	QUANTIFICATION OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRIC METHOD
19.	RECOVERY STUDIES FOR FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRIC METHOD
20.	INTERDAY AND INTRADAY ANALYSIS OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRIC METHOD
21.	RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRIC METHOD
22.	OPTICAL CHARACTERISTICS DAPOXETINE OF BY HPTLC METHOD
23.	QUANTIFICATION OF FORMULATION (PRILYXET 30) BY HPTLC METHOD
24.	RECOVERY STUDIES FOR FORMULATION (PRILYXET 30) BY HPTLC METHOD
25.	INTERDAY AND INTRADAY ANALYSIS OF FORMULATION (PRILYXET 30) BY HPTLC METHOD

LIST OF ABBREVIATIONS USED

ICH	-	International Conference on Harmonization
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
µg/ ml	-	Microgram per Milliliter
mg/ tab	-	Milligram per tablet
ml	-	Millilitre
mM	-	Milli Mole
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% R.S.D	-	Percentage Relative Standard Deviation
S.D	-	Standard Deviation
S.E.	-	Standard Error
UV	-	Ultraviolet
HPTLC	-	High Performance Thin Layer Chromatography
AUC	-	Area under Curve
°C	-	Degree Celsius
Gms	-	Grams
µl	-	Microlitre
rpm	-	Rotations per Minute
µ	-	Micron

v/ v	-	Volume/Volume
IR	-	Infra Red
USP	-	United States of Pharmacopoeia
MBTH	-	3-Methylbenzthiazolinone-2(3H)-Hydrazone

INTRODUCTION

1. INTRODUCTION

1.1 ANALYTICAL CHEMISTRY (Gary.D.Christian, 2007)

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a sub discipline of chemistry has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate composition of new compounds.

Types

Traditionally, analytical chemistry split into two types, Quantitative and Qualitative

Quantitative and Qualitative

Quantitative analysis seeks to establish the amount of a given element or compound in a sample. Qualitative analysis seeks to establish the presence of a given element, given functional group or inorganic or organic compound in a sample

CHEMICAL ANALYSIS CLASSIFIED AS (Skoog, 2005)

1. Clinical analysis
2. Industrial analysis
3. Bio chemical analysis
4. Cosmetic analysis
5. Medicinal analysis
6. Agriculture analysis

MOLECULAR ANALYSIS CLASSIFIED AS (Chatwal, 2006)

1. Nuclear magnetic resonance
2. Intra red spectra copy
3. Ultra violet absorption
4. Ultra violet fluorescence
5. Spectrophotometric analysis
6. X-ray diffraction
7. X-ray absorption
8. Radio tracer techniques
9. Mass spectrometry
10. Thermal analysis
11. Gas chromatography
12. Liquid chromatography
13. Electron microscopy

MORDEN ANALYTICAL CHEMISTRY (Kausture, 2007)

- Involves usage of microprocessor, computers, amplifiers and integrated circuits to make the process.
- The branches of atomic spectroscopy including optical absorption, optical, emission and optical fluorescence, spectrometry, atomic mass spectrometry and atomic x-ray spectrometry.
- The branches of atomic spectroscopy including optical absorption, optical emission, and optical fluorescence spectrometry, atomic mass spectrometry, and atomic x-ray spectrometry.

- In the field of analysis the most important this impossible makes new idea in the pharmaceutical development.
- The accuracy of the calculating value by the most of instrument high qualified like chromatogram, in microgram.
- The tools we need in analytical chemistry to answering the basic knowledge for the type of question.

METHOD DEVELOPMENT

Analytical methods Development and Validation plays an important role in the discovery, development, and Manufacture of Pharmaceuticals

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and Validation of Analytical methods. This presentation will discuss the development and Validation of Analytical method (Spectrophotometric & High performance thin layer chromatography for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

SELECTION OF ANALYTICAL TECHNIQUES (mendham J *et al.*, 2002)

1. Proximate analysis : Determines the amount of each element in a sample.
2. Partial analysis : Determines selected constituents in the sample.

3. Trace constituents analysis : Determines specified components present in very minute quantity.
4. Complete analysis : Determine the portion of each component in the sample.

PHASES IN ANALYTICAL CHEMISTRY (Gary, 2007)

Phase 1: Fast screening phase eg : Immuno assay, gas chromatography and liquid chromatography.

Phase 2 : Identification phase eg : GC – MS.

Phase 3 : Qualification phase eg : Spectrophotometer and gas chromatography.

IMPORTANT CONSIDERATIONS IN ANALYTICAL METHODS (Willard, 1986)

The instrument most visible and exciting element of the analytical method, only one component of the total analysis.

- The analyst should determine the nature of the sample, the end use of the analytical results, the species to be analyzed.
- Quantitative information may include elemental composition, oxidation state, functional groups, major components, minor components, complete identification in the given sample.
- Quantitative data include accuracy and precision, range of expected analyte.
- Methods such as controlling the atmosphere to which the sample is exposed, controlling the temperature of the sample, buffering the pH of sample solutions.

SENSITIVITY AND DETECTION LIMITS (Y.R. Sharma, 2009)

TECHNIQUES	DETECTION LIMITS	IDENTIFICATION LIMITS
Gas chromatography	10^{-6}-10^{12}	-
Infrared	10^{-7}	10^{-6}
spectrophotometry	10^{-7}	10^{-6}
Ultraviolet spectroscopy	10^{-7}	10^{-5}
N.M.R(time averaged)	10^{-6}	10^{-5}
Mass spectrometry (batch inlet)	10^{-12}	10^{-11}
Mass spectrometry (direct probe)		

FACTORS AFFECTING THE ANALYTICAL METHOD

(mendham J *et al.,* 2002)

- Problems arising from the nature of the material to be investigate (eg) radioactive substance affected by water.
- Possible interference from components of the material other than those of interest.
- The concentration range to be investigated.
- The accuracy required.
- The facilities available, particularly the instruments.
- The number of analyses of similar type which have to be performed.

PHYSICAL PROPERTIES USED IN ANALYTICAL CHEMISTRY (Skoog, 2005)

S. No	Physical Property Measured	Instrumental methods based on measurement of property
1.	Absorbtion of radiation	Spectrophotometry
2.	Emission of radiation	Emission spectroscopy, flame photometry, radio chemical method.
3.	Scattering of radiation	Turbidometry, nephelometry
4.	Refraction of radiation	Refractometry, interfero
5.	Diffraction of radiation	X-ray, electron diffraction
6.	Rotation of radiation	Polarimetry, optical rotatory dispersion.
7.	Electrical potential	Potentiometry
8.	Electrical conductance	Conductivity
9.	Quantity of electricity	Coulometer
10.	Mass-to-charge ratio	Mass spectroscopy
11.	Electrical current	Polargraphy
12.	Thermal properties	Thermal conductivity

STAGES IN CHEMICAL ANALYSIS (mendham J *et al.,* 2002)

Evaluation of the satisfactory results

The nature of the physical and chemical system may difficult in analytical chemist. Example, all over the chemist can provide problem of dissolution and separation of a multi-component high temperature among or with separation of a mixture of all

amino acids. He may also be asked to analyse polluted air or the insecticides in fish, birds, plants or animal to measure the rate of reaction, or to determine the number of electrons and intermediates involved in an electro chemical reaction

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any Pharmacopoeias
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients
- Analytical methods for the quantization of the drug in biological fluids may not be available
- Analytical methods for a drug in combination with other drugs may not be available
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable

SPECTROSCOPY (Sharma Y.R, 2009)

Spectrophotometry is based upon the measurement and interpretation of electromagnetic radiation absorbed, when the molecules or atoms or ions of a sample due to electronic transition within the molecule. Every atom, ion or molecule has a unique characteristic relation with electromagnetic radiation

Introduction to Spectrophotometric Methods of Analysis for Drugs in Combination

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods.

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of the samples is known, the identity and concentration of the interfering substance are known and the extent of interference in the assay may be determined.

The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths,

- The absorbance of a solution is the sum of absorbance of the individual components
- The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell
- There are various spectrophotometric methods are available which can be used for the analysis of a combination samples. Following methods can be used Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference Spectrophotometry
- Solvent extraction method

1.2 ULTRAVIOLET SPECTROSCOPY

INTRODUCTION (Kalsi, 2007; Y.R.Sharma, 2009)

Ultraviolet spectroscopy deals with the measurement of energy absorbed when electrons are promoted to higher energy levels. On passing electromagnetic radiation in the ultraviolet and visible regions through a compound with multiple bonds, a portion of the radiation is normally absorbed by the compound. The amount of absorption depends on the wavelength of the radiation and the structure of the compound. Absorption of electromagnetic radiation in the visible and ultraviolet regions of the spectrum resulting in changes in the electronic structure of ions and molecules.

Ultraviolet spectrum records the wavelength of an absorption maximum, i.e. λ_{max} and the strength of the absorption, i.e. molar absorptivity (extinction co-efficient Σ_{max}) as defined by the combined Beer-Lambert law.

The ultraviolet spectrum will be divided into the following region.

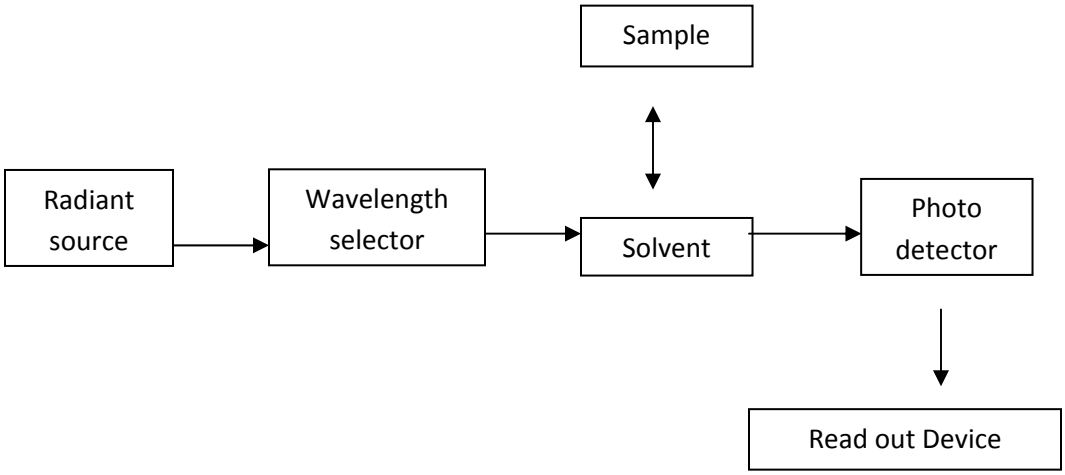
1.Far (or vacuum) ultra violet → 10 – 200 nm

2.Near or quartz ultraviolet → 200 – 380 nm

3.Visible region → 380 – 780 nm

The visible region corresponds to 400-800 nm and ultraviolet region to 200-400 nm.

INSTRUMENTAL MODULES FOR MEASURING ABSORPTION OF UV RADIATION (Willard, 1986)



ABSORPTION SPECTROSCOPY DEPENDS UPON THE FOLLOWING PARAMETERS (A.H.Beckett, and J.B.Stenlake, 2007; Douglas, 2005; Willard, et.al., 1986)

1) TRANSMITTANCE (T)

It is the ratio of intensity of transmitted light to that of incident light

T = It / Io

2) ABSORBANCE (A)

It is negative logarithm of transmittance to the base $_{10}$

$$\mathbf{A = -\log_{10} = \log_{10} Io/ It}$$

$$\mathbf{A = abc}$$

3) MOLAR ABSORPTIVITY (ε)

When concentration ‘’c’’in equation A=abc is expressed in mole/lit and cell length in

‘’cm ‘’ then absorptivity is called as molar absorptivity.

$$\varepsilon = A/bc$$

4) BEER –LAMBER’S LAW (Jagmohan, 2006)

It can be stated as the intensity of beam of monochromatic light. When passed through transparent medium decreases exponentially as the thickness and concentration of absorbing media increases arithmetically.

$$\mathbf{Log_{10}} \left(\frac{I_0}{I} \right) = \sum cl = A$$

Where, I_o - Intensity of incident light

I - Intensity of emerged light

Σ - Molar absorptivity

c - Concentration of solute in moles/litre

l - Path length (Cm)

A - Absorbance

LIMITATIONS OF BEER-LAMBERT’S LAW

The Beer-Lambert law is rigorously obsessed provided a single species gives rise to the observed absorption. However the law may not obsessed when,

i) Different forms of the absorbing molecules are in equilibrium.

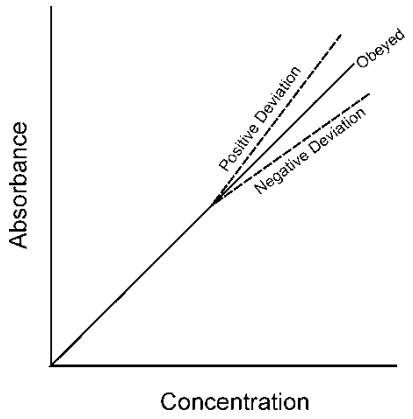
ii) Solute and solvent from association complexes.

iii) There is a thermal equilibrium between ground electronic state and a low lying excited state.

iv) The compounds are charged by irradiation (fluorescent compounds).

DEVIATIONS FROM BEER'S LAW (B.K.Sharma, 2007)

According to Beer's law, a straight line passing through the origin should be obtained, when a graph is plotted between absorbance (A) and concentration. Deviation from the law may be positive or negative, according to whether the resulting curve is concave upwards or concave downwards as shown in the following figure.



The deviations from the Beer's law may be due to interaction of the solute molecules with each other or with the solvent or may be due to instrumental factors.

The most important reasons that cause deviations are

1. Negative deviation can always be expected when the illumination is not monochromatic.
2. The presence of impurities that fluoresces or absorb at the required absorption wavelength.
3. Environmental errors such as solvent, temperature and pressure.

4. Chemical factors such as change in pH and chemical equilibrium, presence of complexing agent, competitive metal ion reactions and concentration dependence.
5. Refractive index of sample.
6. Instrumental errors such as radiation, stability of radiation source, stability of slit control and electronics and reliability of the optical parts.

IMPORTANT CHARACTERSTICS OF SPECTROPHOTOMETRIC AND PHOTOMETRIC METHODS INCLUDE

- Wide applicability to both organic and inorganic systems.
- Typical sensitivities of 10^{-4} to 10^{-5}
- Moderate to high selectivity.
- Good accuracy.
- Ease and convenience of data acquisition.

CHOICE OF SOLVENT (Jagmohan, 2006)

- The solvent used should be of high purity, generally referred to as spectro grade
- Care should be taken to keep lint and dust from contaminating the final solutions
- A good solvent should be transparent over the desired range of wavelengths
- Another factor pertains to the fine structure of an absorption band depends on the polarity of the solvent
- A solvent should be chosen so that it does not react chemically with the sample

SOLVENT EFFECTS (William Kemp, 1996)

The position and intensity of an absorption band may shift when the spectrum is recorded in different solvents. For changes to solvents of increased polarity we can summarize the normal pattern of shifts as follows.

1. Conjugated dienes and aromatic hydrocarbons experience very little solvent shift.
2. α,β unsaturated carbonyl compounds show two different shifts.

   i) The π - π* bond moves to longer wave length (**red shift**)

   ii) The n - π* bond moves to shorter wavelength (**blue shift**)

FOLLOWING TABLE GIVES A LIST OF COMMON SOLVENTS USED IN UV SPECTROSCOPY (Jagmohan 2006)

S.NO	**SOLVENT**	**CUT-OFF WAVELENGTH (λ max nm)**
1.	Acetonitrile	210
2.	Acetone	330
3.	Benzene	280
4.	Benzonitrile	300
5.	Chloroform	245
6.	Carbon tetra chloride	265
7.	Cyclohexane	210
8.	Dioxane	225
9.	Ethanol	210
10.	Ethyl ether	210
11.	Heptane	210
12.	Hexane	210

13.	Iso -octane	210
14.	Methanol	215
15.	n- butanol	210
16.	N-N dimethyl formamide	270
17.	Nitro methane	380
18.	Pyridine	305
19.	Water	210
20.	Xylene	295

WAVELENGTH CHOICE (Robert D Braun, 2006)

When choosing a wavelength at which to make an absorbance measurement, three factors must be considered.

Factor 1

If the solution contains more than one absorbing species, the wavelength that should be chosen, whenever possible, is that at which the second species in the solution does not absorb radiation.

Factor 2

It must be considered when a wave length is chosen is the required sensitive of the assay.

Factor 3

This factor to be considered is the sensitive of the assay to small changes in wave length it is preferable to choose a wavelength at which the absorbance will not be significantly altered if the wavelength is slightly changed.

DETECTORS (B.K. Sharma, 2007; Willard, 1986)

A detector is a transducer that convert electromagnetic radiation into an electron flow and subsequently, into a current flow or voltage in the readout circuit. Photoelectric or photo multiplier tubes are generally used as detectors.

The detector must have the following important requirements.

a) It must respond to radiant energy over a broad wavelength range.
b) It should be sensitive to low levels of radiant power.
c) It should rapidly respond to the radiation and produce an electrical signal that can be readily amplified.
d) It should have relatively low noise level (for stability).
e) The signal produced is directly proportional to the power of beam striking it.

QUALITATIVE ANALYSIS (A.H.Beckett, and J.B.Stenlake, 2007)

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λ_{max}) where small errors in setting the wavelength scale have little effects on the measured absorbance.

QUANTITATIVE ANALYSIS

Absorption spectroscopy is one of the most useful tools to access for the quantitative analysis, the most important characteristics of photometric and spectrophotometric methods are high selectivity and ease of convenience. Quantitative analysis of a single component can be done using following methods.

1. Use of A (1%, 1cm) values.

2. Use of calibration graph (multiple standard methods).

3. By single or double point standardization method.

USE OF A (1%, 1CM) VALUES

This method can be used for the estimation from formulation or raw material when reference standard not available. The use of standard A (1%, 1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorption.

USE OF CALIBRATION GRAPH

In this method absorbance of a number of standard solutions of reference substance at concentrations encompassing sample concentrations are measured, and a calibration graph is constructed. The concentration of analyte in the sample solution read from the graph as concentration corresponding absorbance of the solution. Calibration data is essential if absorbance has nonlinear relationship with concentration if the absorbance values and concentrations bear a linear relationship the regression line then,

$Y = \alpha + \beta x$ may be calculated by method of least squares.

$$\textbf{Intercept } (\alpha) = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma x y)}{N \Sigma x^2 - (\Sigma x)^2}$$

$$\textbf{Slope } (\beta) = \frac{N \Sigma x y - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - (\Sigma x)^2}$$

Where,

y = Absorbance value at concentration x.

N = Number of pairs of values.

The data may be further evaluated statistically to confirm a linear relationship between x and y and provides confidence limits for the slope intercept and estimated concentration of sample.

SINGLE OR DOUBLE POINT STANDARDIZATION

The procedure involves the measurement of the absorbance of a sample solution and of a standard of the reference substance. The standard and the sample solution are prepared in similar manner; ideally the concentration of the standard solution should be close to that of the sample solution. The concentration of the substance in the sample is calculated using following formulas

$$\mathbf{C_{test} = A_{test} / A_{std} \times C_{std}}$$

Where,

C_{test} and C_{std} are the concentration of the sample and standard solutions, respectively.

A_{test} and A_{std} are the absorbance of the sample and standard solutions, respectively.

In double point standardization, the concentration of one of the standard solutions is greater than that of the sample while the other standard solution has a lower concentration than the sample solution. The concentration of the substance in the sample solution is given by

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1} - C_{std2}) + C_{std1}(A_{std1} - A_{std2})}{A_{std1} - A_{std2}}$$

Where,

C_{std} is the concentration of the standard solution.

A_{test} and A_{std} are the absorbance of the sample and standard solutions, respectively. Std 1 and std 2 are the more concentrated standard and less concentrated standard solutions, respectively.

VALIDATION OF ANALYTICAL PARAMETERS IN PHARMACEUTICAL ANALYSIS BY UV SPECTROPHOTOMETRY

1. SENSITIVITY

Knowledge of the sensitivity of the reaction is important and three methods are commonly employed for expressing sensitivity.

Sandell's sensitivity

Number of micrograms of drug converted into the colored product is determined; which in a column solution of cm^2/cross-section shows an absorbance of 0.001 or 0.005 (expressed as µg of drug/cm^2).

Molar extinction co- efficient (∈)

It is calculated from the equation

$$\in = A/IC$$

Where,

A = absorbance

C = Concentration of colored species (Mole/Iit)

I = light path length (cm)

Ringbom plots

Optimum photometric ranges are calculated from the linear portion of the Ringbom plot between Percent transmission Vs Logarithm of the concentration of the drug (expressed as μg/ml).

Knowledge of the above mentioned method is necessary, for comparing the sensitivity of the proposed and reported methods to each drug.

2. CORRELATION COEFFICIENT(r)

When the changes in one variable are associated or followed by changes in the other, it is called correlation. The numerical measure of correlation is called the coefficient of correlation and is defined by the relation.

$$r = \frac{N \varepsilon xy - \varepsilon x . \varepsilon y}{\sqrt{(N \varepsilon x^2 - (\varepsilon x)^2 . N \varepsilon y^2 - (\varepsilon y)^2)}}$$

3. REGRESSION EQUATION

$$Y = mx + c$$

$$m = \text{slope} = \frac{N \varepsilon xy - \varepsilon x . \varepsilon y}{N \varepsilon x^2 - (\varepsilon x)^2}$$

$$c = \text{Intercept} = \frac{\varepsilon y \cdot \varepsilon x^2 - \varepsilon x \cdot \varepsilon xy}{N \varepsilon x^2 - (\varepsilon x)^2}$$

4. PRECISION

The precision (or reproducibility) of the proposed method was ascertained by analyzing the same concentration ($3/4^{th}$ of the upper Beer's law limit) of the drug in freshly prepared test solution eight times. The set of absorbance values obtained were then used to obtain the standard deviation, which is expressed in absorbance units or as a percentage of mean absorbance.

$$S = \sqrt{(X - X)^2/N - 1}$$

Where,

X = observed values

X = Arithmetic mean = $\sqrt{X/N}$

N = Number of deviations

For practical interpretation it is more convenient to express 'S' in terms of percent of the approximate average of the range of analysis is used in the calculation of 'S'. This is called co-efficient of variation (C.V.) or percent relative standard deviation (% R.S.D.).

It is customary to use probability limits (P) 0.05 level (95% of the readings will be within the calculated limits $x \pm a$, where $a = t.s/\sqrt{n}$, t value is 2.365 from Students table) and 0.01 level (99% of the readings will be within the limits $x \pm b$, where $b = t.s/\sqrt{n}$, t value is 3.499 from students Table for eight determinations).

% range of error at p = 0.05 level = $\pm$ 100 a/x

% range of error at p = 0.01 level = $\pm$ 100 a/x

5. ACCURACY

The accuracy of the recommended procedure is evaluated by comparing the values obtained in the proposed and reported methods.

Percent recovery studies

Recovery studies by adding known quantities of drug to previously analyzed pharmaceutical preparations are followed using proposed procedure. To study percent recovery, fixed amount of the sample is taken in a series of volumetric flasks and three different levels of standard solutions are added. Each level of the added drug is repeated six times. The total amount of the drug is then determined by the proposed method.

The percent recovery is calculated by using the equation:

$$\% \text{ Recovery} = \frac{N.\sqrt{xy} - (\sqrt{x})(\sqrt{y})}{N\sqrt{x^2} - (\sqrt{x})^2} \times 100$$

Where

x = amount of drug added in mg/g of sample

y = amount of drug found in mg/g of sample

N = total number of observations

6. INTERFERENCE STUDIES

The effects of a wide range of excipients and other additives usually present in formulations on the determinations under optimum conditions were investigated.

In the initial interference studies, a fixed concentration of the drug was determined several times by the recommended procedure in the presence of a suitable

(1-100 fold) molar excess of the foreign compound under investigation and its effect on the absorbance of solution was noted. The foreign compound was considered to be not interfering at these concentrations if it consistently produces an error less than 3% in the absorbance produced in pure solution.

1.3 AREA UNDER CURVE METHOD (Telekone *et al*., 2010)

The area under curve method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength area is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. In combination drugs λ_1 and λ_2 denotes the wavelength ranges of the components. The integrated value of absorbance in the wavelength ranges of both the drugs are substituted in the simultaneous equation to get the concentration of the drugs.

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \text{ and } c_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

1.4 DERIVATIVE SPECTROSCOPY

(Robert D. Braun, 2006, Beckett *et al*., 2007, Kenneth A Connors, 2002,)

INTRODUCTION

Derivative spectrophotometry is useful means of resolving two overlapping spectra and eliminating matrix interference due to an indistinct shoulder on side of an absorption bands. It involves conversion of normal spectrum [A= f (λ)] to its first [dA/ dλ = f (λ)], second [$d^2A/ d\lambda^2$ = f (λ)] and higher derivatives spectra where the amplitude in the derivative spectrum is proportional to the concentration of the analyte provided that Beer's law is obeyed by the fundamental spectrum.

$$\mathbf{dA/ d\lambda = (dA/dt) / (d\lambda /dt) = (dA/dt)(1/C)}$$

A first derivative spectrum is a plot of dA/dλ or dT/dλ as a function of wavelength, and a second derivative spectrum is a plot of $d^2A/d\lambda^2$ as a function of λ. examples of instruments that are capable of recording derivative spectra include the Baush and Lomb spectronic 2000, the varian DMS 90 and DMS 100, the Gilford 2600, and Hitachi 100-80.

First and second derivative spectra often show detail that is lacking in conventional spectra. That detail can be an aid in the qualitative or quantitative analysis of sample.

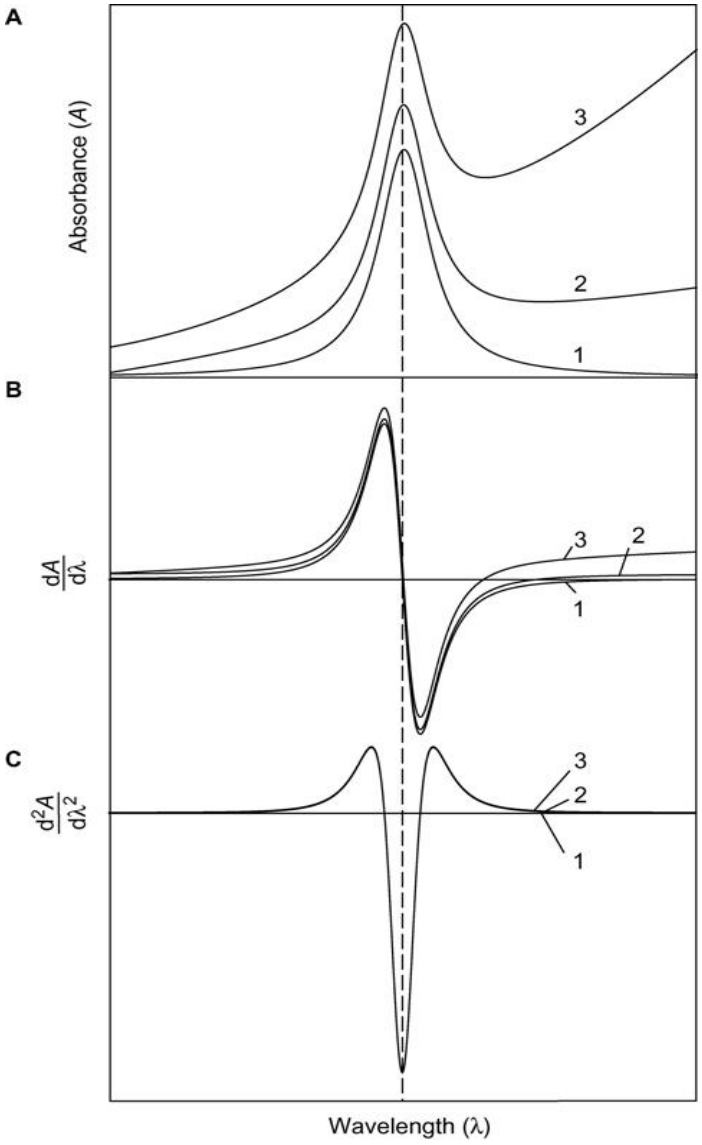
INSTRUMENTATION (Robert D.Braun, 2006)

Derivative spectra may be generated by any of three techniques. The earliest derivative spectra were obtained by modification of the optical system spectrophotometers with dual

monochromators set a small wavelength interval (▲λ, typically 1-3 nm) apart, or with the facility to oscillate the wavelength over a small range, are required

TYPICAL DIAGRAM OF DERIVATIVE SPECTRA

(http://www.medicinescomplete.com)



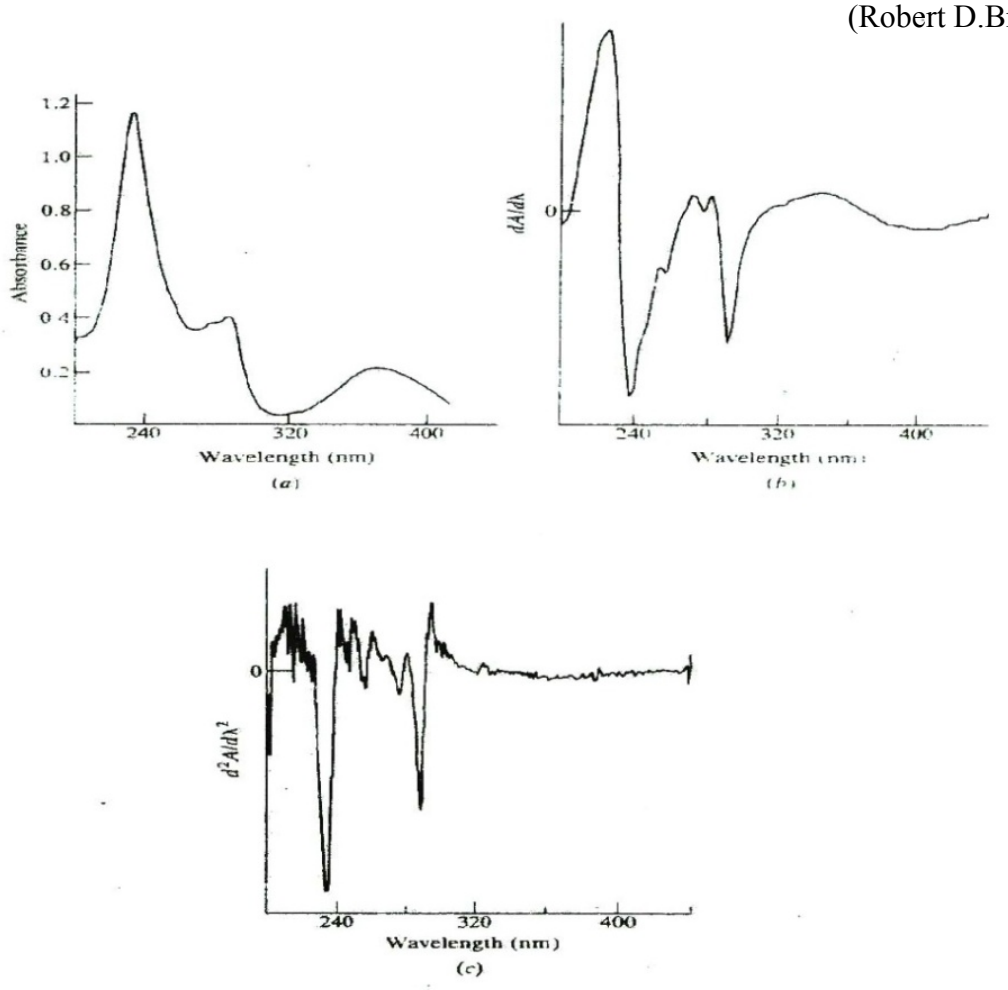
In either case the photo detectors generates a signal with amplitude proportional to the slope of the spectrum over the wavelength interval. Instruments of this type are expensive and are essentially restricted to the recording of first derivative spectra only.

The second technique to generate derivative spectra is electronic differentiation of the spectrophotometers analog signal resistance capacitance (RC) modules may be incorporated in series between the spectrophotometer and recorder to provide differentiation of the absorbance, not with respect to wavelength, but with respect to time, there by producing signal dA/dt.

The third technique is based upon micro computer differentiation. micro computers incorporated into or interfaced with the spectrophotometer may be programmed to provide derivative spectra during or after the scan, to measure derivative amplitudes between specified wavelengths and to calculate concentration and associated statistics from the measured amplitudes.

DIAGRAM OF ZERO, FIRST AND SECOND ORDER SPECTRAS OF A COMPOUND

(Robert D.Braun, 2006)



1.5 VISIBLE SPECTROPHOTOMETRY

(B.K. Sharma, 2007, Mendham J et al., 2002)

Visible spectrophotometry involves the measurement of the amount of visible radiation (400 - 800 nm) absorbed by a colour solution. Using this, the quality of an element present is estimated from the intensity of the colour of the solution due to the presence of a coloured compound of that element. The more intense colour is the higher concentration of the element in solution.

Some compounds are self coloured and for other it is necessary to develop colour by the addition of one or more colour forming reagents (chromogenic reagents) such as MBTH, NED, FC etc. The absorbing capacity of a coloured system is directly proportional to the amount of desired constituent.

Colorimetric analysis should satisfy following criteria.

- The colour reaction should be specific
- Proportionality change between colour and concentration
- Colour should be stable to permit an accurate reading
- Reproducible result should be notified
- Solution must be free from precipitate
- The colour reaction should be highly sensitive

In physics colorimetry refers to the measurement of colour and the determination involves neither the nature of the colourant nor its amounts. In chemistry, a general term called absorptiometry is used for chemical analysis through measurement of radiation. Chemical systems which exhibit a selective light absorptive capacity or coloured, and hence the terms colorimetric analysis and colorimetry are often applied to the

measurements of such system when the object is to determine the concentration of the consistutents responsible for the colour. In other words the variation of some component forms the basis of colorimetric analysis and it is concerned with the determination of the concentration of a substance by measurement of the relative absorption of light with respective of a known concentration of substance.

Colours of different wavelength (kasture *et al.,* 2007)

Wavelength (nm)	Absorbed color	Transmitted color
380-450	Violet	Yellow – green
450-495	Blue	Yellow
495-570	Green	Violet
570-590	Yellow	Blue
590-620	Orange	Green – blue
620-760	Red	Blue – green

Disadvantage of colorimetry

1. These methods always require the availability of a standard or a series of standards.
2. The eye will not be able to match the colour in presence of a standard colored substances. If present in the solution.
3. The eye is not sensitive enough to small differences in absorbances as a photoelectric device.
4. Concentration difference smaller than about 5% relative are difficult to be deteceted.

1.6 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

(sethi P.D.,1996)

Set the analytical objective first that may be quantification or qualitative identification or separation of two components/multicomponent mixtures or optimization of analysis time before starting HPTLC. Method for analyzing drugs in multicomponent dosage forms by HPTLC demands primary knowledge about the nature of the sample, namely, structure, polarity, volatility, stability and the solubility parameter.

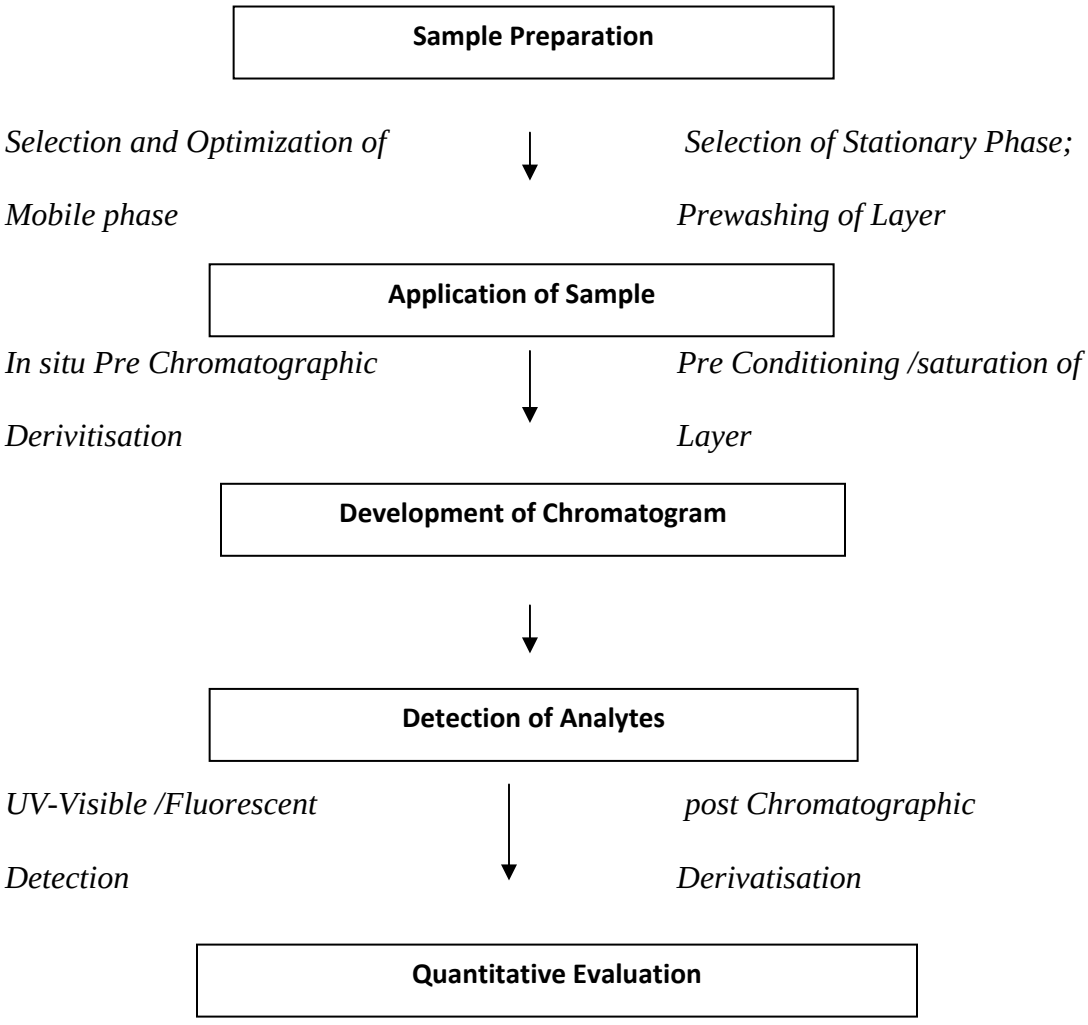
An exact recipe for HPTLC, however, also same like HPLC cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase.

Various process involved in HPTLC

1. Selection of HPTLC plates and solvent.
2. Sample preparation including prechromatogrphic derivitization.
3. Application of sample.
4. Development (separation)
5. Detection including post chromatographic derivitization.
6. Quantification.
7. Documentation.

Method Development for HPTLC

1. Set your analytical objectives –qualitative identification or quantitative
2. Separation of two components or multi component mixtures
3. Collect information about the sample regarding structure, polarity, solubility and volatility.



Plates

Plates are classified as two types

1. Hand made plates.

2. Pre-coated plates.

Mobile phase

Poor grade of solvent used in prerparing mobile phase have found ro decrease resolution, spot definition and R_f reproducibility.

Activation of precoated plates

Plates exposed to high humidity or kept on hand for long time may have to be activated by placing in oven at 110-120˚C for 30 minutes.

Sample prepation

The sample preparation is not as demanding as for other chromatographic techniques, however several steps for sample pre-treatment may be necessary such as sampling, mechanical crushing, extraction, filtration and enrichment of minor compounds. Proper sample preparation is an important for success of HPTLC separation.

Application of sample

The sample should be applied through clean upper end of the capillary and for that filling to marked point, reverse the capillary for applying the sample.

Pre-conditioning (chamber saturation)

If the tank is saturated prior to development, solvent vapours soon get uniformly distributed throught the chamber. As soon as the plate is placed in such a saturated chamber, it soon gets pre-loaded with solvent vapours, hence less solvent shall be required to travel particular distance, resulting lower R_f values.

Development and drying

After development the plate is removed from the chamber and mobile phase is removed as completely and as quickly as possible. This step should preferably be performed infume cup board to avoid contamination of laboratory atmosphere. The plates should always be laid horizontally, so that while mobile phase evaporates the separated substances will migrate evenly to the surface where they can be easily detected.

Detection and visualiztiion

Detection sensitivity depends on the reagent employed. Iodine is the universal detection reagent, the detection usually non-destructive and reversible but certain substance may be altered through non-reversible derivitization.

Quantification

- Sample and standard should be chromatographed on same plate
- After development chromatogram is scanned
- Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode
- Scanning speed is selectable up to 100 mm/s
- Spectra recording are fast - 36 tracks with up to 100 peak windows can be evaluated
- Calibration of single and multiple levels with linear or non-linear regressions are possible when target values are to be verified such as stability testing and dissolution profile single level calibration is suitable
- Statistics such as RSD or CI report automatically
- Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

1.7 VALIDATION OF ANALYTICAL METHOD

(Code Q2A, 1994; Code Q2B, 1996; ICH Guidelines)

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.

When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below

1. Accuracy
2. Precision(Repeatability and Reproducibility),
3. Linearity and range
4. Limit of detection (LOD)/ limit of quantization (LOQ),
5. Selectivity/ specificity
6. Robustness/ ruggedness and
7. Stability and system suitability studies

Advantages of Analytical method Validation:

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.

Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

ANALYTICAL METHOD VALIDATION:

Key Parameters of the Analytical Method Validation:-

It is important for one to understand the parameters or characteristics involved in the validation process. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows.

(1) Accuracy: -

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value.

(2) Precision: -

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at

- Different Occasions,
- Different Laboratories,
- Different Batch of Reagent,
- Different Analysts,
- Different Equipments.

Determination of Repeatability:

It defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is

repeated several time in a standard situation. (RSD below 1% for built drugs, RSD below 2% for assays in finished product).

Determination of reproducibility:

It means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

(3) Linearity and range:

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The linear range of detect ability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y= ax + b together with the correlation coefficient of determination r. For the method to be linear the r value should be close to1.

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

(4) Limit of Detection and limit of Quantitation:

Limit of detection:

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level.

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a) which may be related to LOD and the slope of the calibration curve, b, by

$$\mathbf{LOD = 3\ S_a / b}$$

Limit of quantitation:-

Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

5) Selectivity and Specificity:-

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analyte.

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analyte with and without the addition of the potentially interfering materials. When such components are either unidentified or unavailable, a measure of selectivity can often be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other components.

(6) Robustness and Ruggedness:-

Robustness:

It is an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

Ruggedness:

It is an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental

conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

(7) Stability and System suitability tests:-

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.

System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments.

1.8 PHARMACEUTICAL STATISTICS (Kenneth, 2002, Sundar Rao P.S *et al*, 2006)

Statistical techniques have been widely used in many diverse areas of scientific investigation. Statistical applications have been recognized as crucial to quality control procedure, test, specification and definitions. Principle of modern analytical techniques and skill in their application are necessary attribute of the successful pharmaceutical analyst, thus does not ensure the satisfactory solution of all the problem that may encountered. Some auxiliary knowledge methods those can aid the analyst in designing experiment, collecting data, and interpreting the result.

1.8.1. Linear regression

Linear regression a statistical technique that defines the functional relationship between two variables by best-fitting straight line. Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient 'r', then the best straight line through the data points has to be estimated. This can often be done be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares)

The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable.

To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N \Sigma xy - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - (\Sigma x)^2}$$

and

$$c = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma xy)}{N \Sigma x^{2-(}\Sigma x)^2}$$

1.8.2. Correlation coefficient (r)

It is a procedure commonly used to characterize quantitatively the relationship between variable. Correlation is related to linear regression. To establish whether there is a linear relationship between two variables x_1 and y_1, use Pearson's correlation coefficient r.

$$r = \frac{n \Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n \Sigma x_1^2 - (\Sigma x_1)^2][n \Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where n is the number of data points.

The value of r must lie between +1 and -1, the nearer it is to +1, the greater the probability that a definite linear relationship exists between the variables x and y, values close to +1 indicate positive correlation and values close to -1 indicate negative correlation values of 'r' that tend towards zero indicate that x and y are not linearly related (they made be related in a non-linear fashion).

1.8.3. Standard deviation (SD)

It is commonly used in statistics as a measure of precision statistics as a measure of precision and is more meaningful than is the average deviation. It maybe thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

$$S = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})}{N-1}}$$

Where,

S is standard deviation.

If N is large (50 or more) then of course it is immaterial whether the term in the denomination is N -1 or N

Σ = sum

$\bar{x}$ = Mean or arithmetic average.

$x - \bar{x}$ = deviation of a value from the mean

N = Number of observations

1.8.4. Percentage relative standard deviation (%RSD)

It is also known as coefficient of variation (CV). It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$\text{C V or \% RSD} = \frac{\text{S.D}}{\bar{x}} \times 100$$

Where,

S.D is the standard deviation,

$\bar{x}$ = Mean or arithmetic average.

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

1.8.5. Standard error of mean (SE)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as

$$\text{S.E.} = \frac{\text{S.D.}}{\sqrt{n}}$$

Where,

S.D = Standard deviation

n = number of observations.

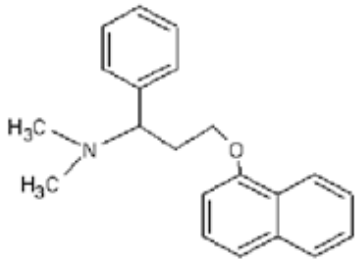
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

DRUG PROFILE (http://en.wikipedia.org/wiki/Dapoxetine.)

DAPOXETINE

MOLECULAR STRUCTURE



CHEMICAL STRUCTURE

N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine

MOLECULAR FORMULA

$C_{21}H_{23}NO$

MOLECULAR WEIGHT

Dapoxetine 341.88g/mol

DESCRIPTION

White to off white powder

STORAGE AND STABILITY

Store at room temperature, protected from light and moisture. Keep out of children.

SOLUBILITY

Soluble in water at 10mg/mL as a free base (pH dependent)

IDENTIFICATION

1. Melting point

Drug	Standard value	Observed value*
Dapoxetine	175-180˚C	175.1

***** average of six determination.

2. IR spectra was recorded and shown in figure 1.

INDICATION

Dapoxetine is indicated for the treatment of premature ejaculation (PE) in men 18 to 64 years of age.

PHARMACODYNAMICS

Dapoxetine is a selective serotonin reuptake inhibitor. Ejaculation is controlled by both serotonin and dopamine: primarily the 5-HT2c and 5-HT1a receptors. It works by inhibiting the reuptake of the serotonin transporter. It also bind to and inhibits the reuptake transporters of dopamine and nor epinephrine.

PHARMACOKINETICS

A randomized, double-blind, placebo-controlled trial of 77 healthy male volunteers was used to assess the pharmacokinetics of Dapoxetine, using a single dose (60, 100, 140, or 160 mg) or multiple doses (60, 100, 140, or 160 mg) of Dapoxetine over a 6 day period. Dapoxetine was rapidly absorbed after being taken by mouth, with a peak plasma concentration between 1.4 and 2.0 hours. This was immediately followed by a rapid decline in plasma concentration, to about 5% of peak concentration within 24 hours.

Distribution

Reported evidence suggests that Dapoxetine is rapidly more than 99% of Dapoxetine is bound to human serum proteins. The active metabolite DesMethylDapoxetine (DED) is 98.5% protein bound. Dapoxetine appears to have a rapid distribution with a mean steady state volume of distribution of 162 L, following values for Dapoxetine were 0, 10, 2, 19 and 19.3 respectively.

Metabolism

The metabolism of Dapoxetine was extensively metabolized to multiple metabolites primarly through the following biotransformational pathways: N-oxidation, N- demethylation, napthyl hydroxylation, glucuronidation and sulfation. There was evidence of presynaptic first pass metabolism after oral administration

Elimination

The metabolite of Dapoxetine were primarily eliminated in the urine as conjugates. Unchanged active substance was not detected in the urine. Dapoxetine has a rapid elimination, as evidence by a low concentration (less than 5% of peak) 24 hours after dosing. There was minimal elimination of Dapoxetine following daily dosing. The terminal half life is approximately 19 hours following oral administration.

DOSAGE AND ADMINISTRATION

The recommended dose for Dapoxetine is 30mg, taken as needed approximately 1 to 3 hour prior to sexual activity. The maximum recommended dosing frequency is once every 24 hours. If the effect of 30mg is insufficient and the side effects are acceptable, the dose may be increased to the maximum recommended dose of 60mg.

Tablets should be swallowed whole to avoid the bitter taste. It is recommended that tablets be taken with at least one full glass of water.

SIDE EFFECTS

Dapoxetine is the only approved medication to treat premature ejaculation. Dapoxetine is well tolerated with very few side effects, the most common side effects: headache, dizziness, nausea or diarrhoea. In case having side effects is not for long term. Dapoxetine is absorbed in body and leaves the body quite quick. Dapoxetine have successfully improved premature ejaculation, including reduction of personal distress and improvement in the satisfaction with the sexual intercourse.

DRUG INTERACTIONS

There are no known drug interactions known to be associated with Dapoxetine at this time. According to published studies, Dapoxetine can be safely taken with the phosphodiesterase (PDE5) inhibitors tadalafil (Cialis) and sildenafil (Viagra).

In patients receiving an SRRI in combination with a monoamine oxidase inhibitor (MAOI), there have been reports of serious, sometimes fatal, reactions including hyperthermia, rigidity, myoclonus, autonomic instability with rapid fluctuations of vital signs and coma.

As with other SSRIs, co-administration with serotonergic medicinal /herbal products may lead to an incidence of serotonin associated effects.

REPORTED METHODS

2.2 REPORTED METHODS

2.2.1 Mehtal Pratik *et al*., (2011) reported **"Development and validation of a RP-HPLC method for the determination of Dapoxetine Hydrochloride in pharmaceutical formulation using an experimental design"**.

A rapid and sensitive RP-HPLC method with UV detection (230 nm) for routine analysis of Dapoxetine HCl in a pharmaceutical formulation (Priligy) was developed. Chromatography was performed with mobile phase containing a mixture of buffer [Tri-ethyl amine, pH-4.0 (adjusted with o-phosphoric acid)] and acetonitrile (60:40, v/v) with flow rate was 1.0ml min-1. The procedure was validated for linearity (correlation coefficient=0.9998), accuracy, robustness and intermediated precision. The R.S.D.value (0.5%, n=6) indicated a good precision of the analytical method. The proposed method was simple, highly sensitive, precise and accurate and retention time less than 5min indicating that the method is useful for routine quality control.

2.2.2 Thyseen AN *et al*., (2010) reported **"Pharmcokinetics of Dapoxetine Hydrochloride in Healthy Chinese, Japanese, and Caucasian Men"**.

Dapoxetine is the first oral medication specifically developed for the on-demand treatment of premature ejaculation. Premature ejaculation is a sexual dysfunction characterized by an inability to control ejaculation, a short intravaginal ejaculatory latency time, and the consequential negative impact of the condition such as low satisfaction with sexual intercourse, distress, and interpersonal difficulty. Dapoxetine is approved in some European Union countries, as well as Mexico and Korea, for the treatment of premature ejaculation.

AIM
AND
PLAN OF WORK

3. AIM AND PLAN OF WORK

3.1 Aim of work

Most of the pharmaceutical industries are manufacture new drug formulations to meet the market requirement. The survey of the Dapoxetine is chemically (S)-N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine. The Dapoxetine is launched and indicated for the treatment of premature ejaculation (PE).

The literature review revealed that there are only few methods reported for quantitative analysis of Dapoxetine in HPLC method. But there is no spectrophotometric method was reported for the estimation of Dapoxetine in tablet dosage form. Plan was targeted to presume the present research project work by selecting Dapoxetine as drug.

3.2 Plan of work

3.2.1 Survey of the literature

From the survey of the literature for Dapoxetine, their physical properties, chemical properties, pharmacological and pharmacokinetic studies details were collected for the development of several analytical methods.

3.2.2 Procurement of drug sample

The Dapoxetine was obtained as gift sample Alkem Laboratories. The commercial formulation was procured from local pharmacy.

3.2.3 Validation of developed methods

All the methods, which have been developed and validated according to ICH guidelines.

Therefore in the proposed research project a successful effort has been made to develop simple economic accurate and rapid methods for the estimation of Dapoxetine in bulk and in tablet dosage formulation and validated all the methods as per ICH guidelines.

The methods are

UV Spectrophotometric Method

Area Under Curve Method

Derivative Spectrophotometric Method

Visible Spectrophotometric Method

High Performance Thin Liquid Chromatography

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Drug Samples (Raw material)

Dapoxetine was obtained as a gift sample from Alkem Laboratories, Hyderabad.

4.1.2 Formulation used

PRILYXET tablets (Sun pharma sikkim, East sikkim) containing Dapoxetine 30 mg was procured from Medplus pharmacy, Chennai.

4.1.3 Chemicals and solvents used

Distilled water, MBTH reagent (AR Grade), Cerric Ammonium Sulphate (AR Grade), Methanol (HPLC grade), Water (HPLC grade), were purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited, Mumbai.

4.1.4 Instruments used

Different instruments used to carry out the present work,

1) Shimadzu AUX - 220 Digital balance
2) Shimadzu - 1700 Double Beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells
3) ELICO SL - 210 Double Beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells
4) ELICO pH meter (Model LI - 120)
5) Sonicator – Sonica ultrasonic cleaner – Model 2200 MH
6) REMI – Centrifuge apparatus
7) CYBERLAB – Micropipette
8) SUNBIM – Melting point apparatus

Specifications (Terms) of instruments

a) Shimadzu AUX - 220 Digital balance (Shimadzu Instruction Manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

b) Double Beam UV - Visible Spectrophotometer (Shimadzu and ELICO Instruction Manual)

Model: Shimadzu UV - 1700; Double beam UV - Visible spectrophotometer.

ELICO SL – 210; Double beam UV - Visible spectrophotometer.

Specification	**Shimadzu UV - 1700**	**Elico SL - 210**
Light source	20 W halogen lamp, Deuterium lamp, Light source position automatic adjustment mechanism. Built in lamp lighting time display function.	Tungsten halogen lamp (W), Deuterium lamp (D), Light source position automatic adjustment mechanism.

Monochromator	Aberration- correcting concave blazed holographic grating	Concave holographic grating with 1200 lines/ mm
Detector	Silicon photodiode	Photodiode
Stray Light	0.04% or less (220 nm; NAI 10g/ lt) 0.04% or less (340 nm; $NaNo_2$ 50g/ lt).	< 0.05% T at 220 nm with NAI 10g/ lt
Measurement Wavelength range	190 ~ 1100 nm	190 ~1100 nm
Spectral Band width	1 nm or less (190 to 900nm).	1.8 nm
Wave length Accuracy	± 0.5 nm on broad automatic wavelength calibration mechanism.	± 0.5 nm automatic wavelength calibration mechanism.

Recording range	Absorbance - 3.99 ~ 3.99 Abs Transmittance; - 399 ~ 399%	Absorbance; ± 3.000 Abs
Photometric accuracy	± 0.004 Abs (at 1.0 Abs). ± 0.002 Abs (at 0.5 Abs).	± 0.005 Abs (at 1.0 Abs). ± 0.010 Abs (at 0.5 Abs).
Operating Temperature/ Humidity	Temperature range; 15 to 35˚C Humidity range; 35 to 80%(15 to below 35˚C) 35 to 70%(30 to below 35˚C)	Temperature range; 15 to 35˚C Humidity range; 35 to 80%(15 to below 35˚C) 35 to 70%(30 to below 35˚C)

4.2 HPTLC

4.2.1 Instrument specification

4.2.2 Stationary phase

Plate size (x×y) - 20×10 cm

Material - HPTLC plates silica gel 60 F 254

Pre washing - No

Modification - No

4.2.3 Sample application – CAMAG Automatic 4

Instrument - CAMAG Automatic TLC sampler 4 (ATS4)

4.2.4 ATS 4 application parameters

Spray gas	-	Nitrogen
Sample solvent type	-	Methanol
Rinsing solvent	-	Unheated
Rack in use	-	standard

4.2.5 Sequence

Syringe size	-	100 µL
Application type	-	Band
Band length	-	8.0 mm

4.2.6 Development – glass tank

Chamber type	-	twin trough chamber 20×10 cm
Pre-conditioning		
Mobile phase	-	Acetonitrile : Ethyl acetate (9 : 1)
Solvent front position	-	50.0 nm
Volume	-	10.0 mL
Drying device	-	Hair dryer
Time	-	5 minutes

4.2.7 Detection – CAMAG TLC SCANNER 3

4.2.8 Instrument – CAMAG TLC scanner 3'' scanner 3_ 140703''

Position of first tract X	-	15.0 mm
Distance between tracks	-	10.5 mm
Scan start position Y	-	10.0 mm
Scan end position Y	-	80.0 mm
Optimize optical system	-	Light
Scanning speed	-	20 mm/s
Data resolution	-	100 µm/step

4.2.9 Measurement table

Wave length	-	292 nm
Lamp	-	D2&w
Measurement type	-	Remission
Measurement mode	-	Absorption
Optical filter	-	Secound order
Dectector mode	-	Automatic
PM high voltage	-	347 V

4.2.10 Integration

Data filtering	-	Savitsy-golay 7
Base line correction	-	Lowest slope
Peak threshold min.slope	-	5
Peak threshold min.height	-	10AU
Peak threshold min.area	-	50
Peak threshold max.height	-	990AU
Track start position	-	46.5
Track end position	-	56.9
Display scaling	-	Automatic (53)

4.2.11 Reagent and chemicals

Methanol gradient HPLC grade

Chloroform HPLC grade

Ethyl acetate HPLC grade

Silica gel GF 254 pre coated plates

Active pharmaceutical ingredients and formulation

METHODS

4.2 METHODS

The estimation of Dapoxetine was done by following methods

4.2.1 UV spectrophotometric method

4.2.2 Area under curve method

4.2.3 Derivative spectrophotometric method

4.2.4 Visible spectrophotometric method

4.2.5 HPTLC method.

4.2.1 UV SPECTROPHOTOMETRIC METHOD

4.2.1.1 Selection of solvent

The solubility of Dapoxetine was determined by using variety of solvents. Solubility study for Dapoxetine was carried out in polar and non polar solvents. The drug was found to be soluble in distilled water. Considering the economic factor, distilled water was selected as the solvent for further analysis.

4.2.1.2 Preparation of standard stock solution

10mg of Dapoxetine working standard was accurately weighed and transferred into a 10 mL of volumetric flask and dissolved in minimum quantity of distilled water and made upto 10mL distilled water. The solution was observed to contain 1000 µg/mL of Dapoxetine.

4.2.1.3 Selection of λmax

The standard stock solution was diluted with distilled water to get a concentration of 10 µg/mL of Dapoxetine. The solution was scanned between 200 and 400 nm range using distilled water as blank. From the UV spectra obtained, 292nm was selected as λmax found to be stable for 4 hours and 30 minutes.

4.2.1.4 Preparation of working standard solution

An accurately weighed quantity of Dapoxetine (10mg) was dissolved in appropriate quantity of distilled water and transferred in a 100 mL volumetric flask, and then volume was made up with distilled water up to the mark to get 100µg/mL.

A standard stock solution was further diluted in a 10 mL volumetric flask with distilled water to get final solution of concentration 10 µg/mL.

4.2.1.5 Linearity and calibration graph

To series of six 10mL volumetric flask, different aliquots (0.5 to 3 ml) were taken from the above working standard solution to prepare a serious of concentration range from (5-30 µg/mL) using distilled water. Calibration curve was prepared at λmax 292 nm against water as blank in the concentration range from 5 to 30 µg/mL.

4.2.1.6 Quantification in formulation

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 25mg Dapoxetine was weighed and transferred to 25ml volumetric flask. Dissolved with distilled water and sonicated for 15 minutes. The solution was made upto 25ml with distilled water filtered through Whatmann filter paper No.41 and pipette out 7.5ml and make up to 50ml with distilled water from that 1ml was pippeted out and made up to 10ml standard flask to get the concentration of 15µg/mL. The absorbance was measured at 292 nm against distilled water as blank. The absorbance measurements were made six times for formulation. The amount of Dapoxetine present in formulation was calculated from the slope and intercept of respective calibration curve.

4.2.1.7 Recovery studies

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of Dapoxetine working standard to the pre-analyzed formulation. To the

50% of pre-analyzed tablet powder equivalent to 25mg (Label claim of tablet), known quantities of standard drug (80, 100 and 120 % of quantification concentration) were added, dissolved with distilled water and made up to volume with distilled water. The solutions were sonicated for 15 minutes. After sonication the solutions were filtered through Whatmann filter paper No 41. The absorbance of the resulting solutions were measured at 292nm against distilled water as blank and the amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times at each level.

4.2.1.8 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The calibration graph was prepared from the serial dilution of the standard solutions were repeated for six times. The Limit of Detection and Limit of Quantification were calculated by using the average of slope and of intercept values.

4.2.1.9 Ruggedness

Ruggedness of the method was performed by precision analysis of formulation was done,

1. By using different analysts.
2. By using different instruments.

The Amount, SD, %RSD and Standard Error was calculated.

4.2.2 AREA UNDER CURVE METHOD

4.2.2.1 Selection of solvent

The solubility of Dapoxetine was determined by using variety of solvents. Solubility studies for Dapoxetine were carried out in polar and non polar solvents. The drug was found to be soluble in distilled water. Considering the economic factor, distilled water was selected as the solvent for further analysis.

4.2.2.2 Preparation of standard stock solution

10mg of Dapoxetine working standard was accurately weighed and transferred into a 10 mL of volumetric flask and dissolved in minimum quality of distilled water and made upto 10mL distilled water. The solution was observed to contain 1000 µg/mL of Dapoxetine.

4.2.2.3 Selection of λmax

The standard stock solution was diluted with distilled water to get a concentration of 10 µg/mL of Dapoxetine. The solution was scanned between 200 and 400 nm range using distilled water as blank. From the UV spectra obtained, Area under curve was calculated between 281.5 nm to 295.5 nm and found to be stable for 4 hours and 30 minutes.

4.2.2.4 Preparation of working standard solution

An accurately weighed quantity of Dapoxetine (10mg) was dissolved in appropriate quantity of distilled water and transferred in a 100 mL volumetric flask, and then volume was made up with distilled water up to the mark to get 100 µg/mL.

A standard stock solution was further diluted to 10 mL volumetric flask with distilled water to get final solution of concentration 10 µg/mL.

4.2.2.5 Linearity and calibration graph

To series of six 10mL volumetric flask, different aliquots (0.5 to 3 ml) were taken from the above working standard solution to prepare a serious of concentration range from (5-30 µg/mL) using distilled water. The area absorbance were scanned at a wavelength of 281.5 nm - 295.5 nm against distilled water as blank. The calibration curve was plotted between concentration and absorbance in the concentration range from 5 to 30 µg/mL and it obeyed Beer's law.

4.2.2.6 Quantification in formulation

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 25mg Dapoxetine was weighed and transferred to 25ml volumetric flask. Dissolved with distilled water and sonicated for 15 minutes. The solution was made upto 25ml with distilled water filtered through Whatmann filter paper No.41and from the above solution pipette out 7.5ml and made up to 50ml with distilled water from that 1ml was pippeted out and made up to 10ml standard flask to get the concentration of 15µg/mL. The absorbance was measured between 281.5 nm - 295.5 nm against distilled water as blank. The absorbance measurements were made six times for formulation. The amount of Dapoxetine present in formulation was calculated from the slope and intercept of respective calibration curve.

4.2.2.7 Recovery studies

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery analysis was done by adding known concentration of Dapoxetine working standard to the pre analyzed formulation. To the 50% of pre-analyzed tablet powder equivalent to 25mg (labeled claim of tablet), known quantities of standard drug (80,100 and 120 % of quantification concentration) were added, dissolved with distilled water and made up to volume with distilled water. The solutions were sonicated for 15 minutes. After sonication the solutions were filtered through Whatmann filter paper No 41. The absorbance of the resulting solutions were measured between 281.5 nm - 295.5 nm against distilled water as blank and the amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times at each level.

4.2.2.8 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The calibration graph was prepared from the serial dilution of the standard solutions were repeated for six times. The Limit of Detection and Limit of Quantification were calculated by using the average of slope and of intercept values.

4.2.2.9 Ruggedness

Ruggedness of the method was performed by precision analysis of formulation was done,

1. By using different analysts.
2. By using different instruments.

   The Amount, SD,%RSD and Standard Error was calculated.

4.2.3 DERIVATIVE SPECTROPHOTOMETRIC METHOD

4.2.3.1 Selection of solvent

From the solubility studies of Dapoxetine, distilled water was selected as a solvent for Derivative spectrophotometric method, by derivatising the zero order spectra to a second order derivative spectrum, showed the maxima 241.0 nm and minima 234.5 nm respectively.

4.2.3.2 Preparation of standard stock solution

10mg of Dapoxetine working standard was accurately weighed and transferred into a 10 mL of volumetric flask and dissolved in minimum quantity of distilled water and made upto 10mL distilledwater. The solution was observed to contain 1000 µg/mL of Dapoxetine.

4.2.3.3 Selection of λmax

The standard stock solution was diluted with distilled water to get a concentration of 10 µg/mL of Dapoxetine. The solution was scanned between 200 and 400 nm range using distilled water as blank. The zero order spectrum was derivatised to zero order spectrum with maxima 241.0 nm and minima 234.5nm respectively. The absorbances of same solution were measured repeatedly for stability study. From the stability studies, Dapoxetine in water was found to be stable for 4 hours and 30 minutes.

4.2.3.4 Preparation of working standard solution

An accurately weighed quantity of Dapoxetine (10mg) was dissolved in appropriate quantity of distilled water and transferred in a 100 mL volumetric flask, and then volume was made up with distilled water up to the mark to get 100 µg/mL. A standard stock solution was further diluted with distilled water to get final concentration 10 µg/mL.

4.2.3.5 Linearity and calibration graph

To series of six 10mL volumetric flask, different aliquots (0.5 to 3 ml) were taken from the above working standard solution to prepare a serious of concentration range from (5-30 µg/mL) using distilled water. Calibration curve was prepared at 234.5 nm and 241.0 nm against water as blank. The calibration curve was plotted between concentration versus absorbance in the concentration range from 5 to 30 µg/mL.

4.2.3.6 Quantification in formulation

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 25mg Dapoxetine was weighed and transferred to 25ml volumetric flask. Dissolved with distilled water and sonicated for 15 minutes. The solution was made upto 25ml with distilled water filtered through whatmann filter paper no.41and from the above solution pipetted out 7.5ml and made up to 50ml with distilled water from that 1ml was pipetted out and made up to 10ml standard flask to get the concentration of 15µg/mL. Then by converting the zero order spectra to second order derivative spectra, the absorbance measurements were made for formulation at minima 234.5 nm and

maxima 241.0 nm using water as blank. The amount of Dapoxetine present in formulation was calculated from the slope and intercept of respective calibration curve.

4.2.3.7 Recovery studies

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of Dapoxetine working standard to the pre analyzed formulation. To the 50% of pre analyzed tablet powder equivalent to 25mg (labeled claim of tablet), known quantities of standard drug (80,100 and 120 %) of quantification concentration were added, dissolved with distilled water and made up to volume with distilled water. The solutions were sonicated for 15 minutes. After sonication the solutions were filtered through Whatmann filter paper No 41. Then by converting the zero order spectra to second order derivative spectra, the absorbance measurements were made for formulation at minima 234.5 nm and maxima 241.0 nm using water as blank. The amount of Dapoxetine present in formulation was calculated from the slope and intercept values. The procedure was repeated for three times at each level.

4.2.3.8 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The calibration graph was prepared from the serial dilution of the standard solutions were repeated for six times. The Limit of Detection and Limit of Quantification were calculated by using the average of slope and of intercept values.

4.2.3.9 Ruggedness

Ruggedness of the method was performed by precision analysis,

1. By using different analysts.
2. By using different instruments.

The Amount, SD, %RSD and Standard Error was calculated.

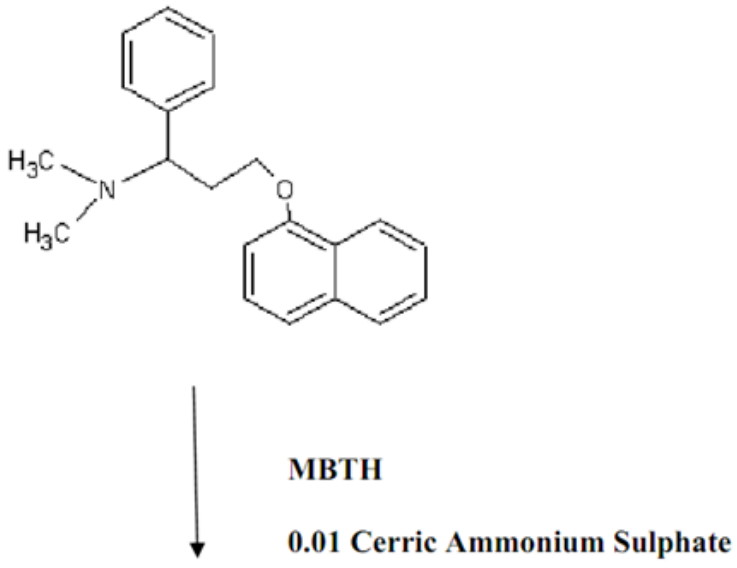
4.2.4 VISIBLE SPECTROPHOTOMETRIC METHOD

4.2.4.1 Principle

Dapoxetine has tertiary amino group, hence it was planned to treat with 0.01M Cerric Ammonium Sulphate and the resulting solution was tested for various color reactions with different chromogenic regents like MBTH, $Fecl_3$, PDAB, FC. Where MBTH reagent gave a stable colour (wavelength of 400- 800nm) with Dapoxetine in presence oxidizing agent (0.01M Cerric Ammonium Sulphate) and have good linearity and obeys Beer's Lambert law.

$$A=abc$$

[A= absorbance, a= absorptivity, b= pathlength and c= concentration]



4.2.4.2 Method development

4.2.4.2.1 Preparation of reagents

1. MBTH

Weighed accurately about 0.2 gm of MBTH and dissolved in 100ml of distilled water.

2. 0.01 M Cerric Ammonium Sulphate

Weighed accurately 0.632 gm of CAS in 100 ml of 1M Sulphuric acid.

3. 1 M Sulphric acid

57 ml of concentrated Sulphuric acid in 1000ml.

4.2.4.3 Optimization of MBTH reagent

Dapoxetine was found to yield a purple colored product with MBTH reagent and 0.01 M Cerric Ammonium Sulphate and has absorbance maximum of 437 nm. Therefore studies were carried out to establish the mostly favorable condition for the formulation of the colored products.

The influence of the concentration with constant volumes of the reagent on the reaction has been studied. Various concentration of MBTH reagent like 1mL, 2mL, 3mL, 4mL, and 5mL were tried by varying the parameter of 0.01M CAS concentration like 1mL, 2mL, 3mL, 4mL, and 5mL at a time, it was found to be 2mL and that of MBTH reagent was found to be 2mL.

The optimum concentration was selected on the basis of their ability to give maximum absorbance. The color was found to stable upto 2 hours.

4.2.4.4 Preparation of standard stock solution

10mg of Dapoxetine working standard was accurately weighed and transferred into a 10 mL of volumetric flask and dissolved in minimum quality of distilled water and made upto 10mL distilled water. The solution was observed to contain 1000 µg/mL of Dapoxetine.

4.2.4.5 Selection of λmax

To 1 mL standard stock solution, added 2 mL of 0.01M Cerric Ammonium Sulphate and 2 mL of MBTH reagent in a 10 mL standard flask and the volume was made up to mark with distilled water. The solution was scanned between 400 to 800nm using reagent as blank. From the visible spectrum obtained, 437 nm was selected as λmax for the analysis of Dapoxetine. The color was found to be stable for 2 hours.

4.2.4.6 Preparation of working standard solution

An accurately weighed quantity of Dapoxetine (10mg) was dissolved in appropriate quantity of distilled water and transferred in a 100 mL volumetric flask, and then volume was made up with distilled water up to the mark to get 100µg/mL. A standard stock solution was further diluted with distilled water to get final solution of concentration 10 µg/mL.

4.2.4.7 Linearity and calibration graph

In this method, the aliquots of working standards (0.5- 3 mL of 100 µg/mL) were transferred into six 10 mL standard flasks, added 2mL of 0.01M Cerric Ammonium Sulphate and 2 mL of MBTH reagent, mixed well and allowed to stand for 5 min.

The solutions were made up to mark with the distilled water. The absorbance measurements were made at 437 nm against reagent as blank. The solutions were found to be linear from 5-30 µg/mL and the calibration curve was plotted between and absorbance.

4.2.4.8 Quantification of formulation

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 25mg Dapoxetine was weighed and transferred to 25ml volumetric flask. Dissolved with distilled water and sonicated for 15 minutes. The solution was made upto 25ml with distilled water filtered through Whatmann filter paper No.41and from the above solution pipette out 7.5ml and made up to 50ml with distilled water from that 1 mL was pipetted out from the above solution and transferred into a 10 mL standard flask then added 2 mL Cerric Ammonium Sulphate and 2mL MBTH reagent. Volume was made up to mark with distilled water to get 15 µg/mL solution. The absorbance was measured at 437 nm using reagent as blank. The amount of Dapoxetine present in the formulation was calculated from the slope and intercept value of the calibration curve.

4.2.4.9 Recovery studies

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of Dapoxetine working standard to the pre analyzed formulation. To the 50% of pre analyzed tablet powder equivalent to 25mg (labeled claim of tablet), known quantities of standard drug (80,100 and120 % of quantification concentration) were added, dissolved with distilled water and made up to volume with distilled water and

added 2mL of 0.01 M Cerric Ammonium Sulphate and 2 mL of MBTH reagent, mixed well and allowed to stand for 5 min. The solutions were made up to mark with the distilled water and filtered through whatmann filter paper No.41. The absorbance were made at 437 nm against reagent as blank. The amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times at each level.

4.2.4.10 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The calibration graph was prepared from the serial dilution of the standard solutions were repeated for six times. The Limit of Detection and Limit of Quantification were calculated by using the average of slope and of intercept values.

4.2.4.11 Ruggedness

Ruggedness of the method was performed by precision analysis,

1. By using different analysts.
2. By using different instruments.

The Amount, SD, %RSD and Standard Error was calculated.

4.2.5 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD

4.2.5.1 Choice of mobile phase

Intial trials were made with prepared TLC plates and pre-coated sheets, the spots were identified using shorter wavelength in UV chamber and they are confirmed by scanning in HPTLC scanner and the individual standards are also spotted to determine the R_f of each drugs.

TRAIL NO	MOBILE PHASE	RATIO
1.	Chloroform : Ammonia : Methanol	6 : 1 : 3
2.	Chloroform : Methanol : Acetic acid	6 : 3 : 1
3.	Acetonitile : Ethyl acetate	9 : 1
4.	Butanol : Acetic acid : Water	5 : 3 : 2
5.	Chloroform : Methanol : Water	6 : 3 : 1

The mobile phase chosen after trail was Acetonitrile : Ethyl acetate due to its better resolution.

4.2.5.2 Detection of wavelength

10 µg/ml of Dapoxetine were prepared and the spectra were recorded in the range of 200 – 400 nm, from the spectra 292 nm was selected as detection wavelength. Where the drug showed maximum absorbance.

4.2.5.3 Optimized chromatographic conditions

Sample prepared in	-	Methanol
Stationary phase	-	silica gel 60 F 254
Mobile phase	-	Acetonitrile : Ethyl acetate (9 : 1)
Scanning wavelength	-	UV 292 nm
Development mode	-	ascending mode

4.2.5.4 Preparation of standard stock solution

A standard stock solution of Dapoxetine was prepared by dissolving in methanol to produce 100 µg/mL and solution was used to establish linearity.

4.2.5.5 Evaluation of linearity

From stock solution of 100 µg/mL, from this 1-6 mL were pipetted out into a series of six 10mL volumetric flask, a final concentration of Dapoxetine ranging from 1 to 6 µg/mL was obtained and this solution was spotted on a pre-coated TLC plates and developed as per the procedure discussed the peak area obtained for the different concentration.

4.2.5.6 Calibration graph

A graph of peak area against concentration was constructed for Dapoxetine in the concentration range of 1 to 6 µg/mL and it was found to be linear.

4.2.5.7 Analysis of sample

Twenty tablets of formulation (Prilyxet 30) containing 30 mg of Dapoxetine were accurately weighed and the average weight was found and powdered. The powdered tablet equivalent to 10 mg of Dapoxetine was transferred into a 100 mL volumetric flask, added 25 mL of methanol and sonicated for 15min, then shaken vigorously for few min and finally made up to the mark with methanol. The above solution was collected by filtering it through Whatmann filter paper No.41. from the filtered solution, 3 µg/mL was spotted on a TLC aluminium sheets silica gel 60 F 254 plates are allowed to develop in twin trough chamber 20x10 cm using Acetonitrile : Ethyl acetate, the solvent front position is noted, the plates are then removed and allowed it to dry in hair drier for 5 min.

The spots are then detected using Camag TLC scanner 3 and the peak area obtained at the detecting wavelength 292 nm, the amount of Dapoxetine was calculated using the regression equation.

4.2.5.8 Recovery studies

The recovery studies were carried out by adding a known quantity of the standard drug to the pre-analysed formulation and the whole contents was re-analysed by the proposed method. The percentage recovery was calculated and the data was tabulated.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Six methods were performed and developed for the estimation of Dapoxetine in bulk pure form. The methods are employed for analysis of Dapoxetine were,

5.1 UV spectrophotometric method

5.2 Area under curve method

5.3 Derivative spectrophotometric method

5.4 Visible spectrophotometric method

5.5 HPTLC method.

5.1 UV SPECTROPHOTOMETRIC METHOD

The solubility status of Dapoxetine was performed as per Indian Pharmacopeia. The various polar and non-polar solvents were carried out for Dapoxetine were Distilled water, Methanol, Ethanol, 0.1 M Hydrochloric acid, Acetone, Ethyl acetate, Isopropyl alcohol, Acetonitrile, Dimethyl formamide, Diethyl ether, Chloroform, Carbon tetra chloride.

From the solubility studies, the Dapoxetine was found to be freely soluble in methanol and acetonitrile, ethyl acetate. Dapoxetine soluble in distilled water, acetone, and dimethyl formamide. It is insoluble in chloroform, carbon tetra chloride, 0.1 M Hydrochloric acid.

Dapoxetine is determined by dissolving in minimum quantity of distilled water and made further dilution with distilled water. The solution was scanned between wavelength range of 200 and 400 nm in the UV region using distilled water as a blank. From the UV spectrum obtained, 292 nm was chosen as maximum wavelength for further analysis of Dapoxetine. The UV spectrum are shown in figure 2.

By using the selected wavelength, the absorbance of solution of was observed and measured in different frequent intervals. It was found that the Dapoxetine having the stability for 4 hours and 30 minutes. So the same wavelength was selected for further analysis.

The different aliquots of Dapoxetine in distilled water were prepared in the concentration range from 5-30 µg/mL. The absorbance of the same solution was measured at 292 nm. The linearity procedure was repeated for six times. From the

linearity analysis, the calibration curve was plotted by using concentration against absorbance. The optical characteristics of Dapoxetine are correlation coefficient, regression equation, slope, intercept, limit of detection, limit of quantification, sandell's sensitivity and standard error of mean were calculated. The reports are shown in the table 2. The average value of correlation coefficient from linearity studies was found to be 0.9999. It mentioned that the concentration of Dapoxetine has good linearity. The calibration graph were shown in figure 6.

The amount of Dapoxetine was determined in the test solution by using average values of slope and intercept. Repeated the same procedure for six times. The average percentage label claim of Dapoxetine present in tablet formulation (Prilyxet 30) was found to be 100.6 ± 0.428657. The percentage relative standard deviation value revealed that the proposed method has good precision. The results of this analysis are shown in table 3.

The precision of the proposed method was further ensured by carry out the recovery analysis. To the 50% of pre-analyzed tablet formulation, a known quantities of Dapoxetine standard solution was added at different levels (80%, 100% and 120% of quantification concentration). The absorbance of the recovery solutions were measured at 292 nm and the percentage purity of recovery analysis were found to be 100.03%.

It was revealed that the excipients did not interface in the tablet formulation of Dapoxetine. The recovery analysis are shown in table 4.

Further, the precision of the proposed method was confirmed by intraday and interday analysis of quantification studies, the quantification analysis was performed for three times in the same day and one time in the three successive days.

The %RSD values were found to be 0.210207 and 0.013981 for intraday and interday analysis of formulation, the low percentage RSD revealed that the precision of the proposed method was ensured. The results of interday and intraday analysis are shown in table 5.

The method was developed and validated by ruggedness analysis. It was performed by different days which may consists different analysts and different instruments. The % RSD values were found to be 1.559669 and 0.743303 for different instruments and different analysts respectively. The reports of ruggedness analysis were shown in table 6. Hence the precision was ensured by ruggedness analysis.

5.2 AREA UNDER THE CURVE METHOD

The solubility status of Dapoxetine was performed as per Indian Pharmacopeia. The various polar and non-polar solvents were carried out for Dapoxetine were Distilled water, methanol, ethanol, 0.1M Hydrochloric acid, acetone, ethyl acetate, isopropyl alcohol, acetonitrile, dimethyl formamide, diethyl ether, chloroform, carbon tetra chloride.

From the solubility studies, the Dapoxetine was found to be freely soluble in methanol and acetonitrile, ethyl acetate. Dapoxetine soluble in distilled water, acetone, and dimethyl formamide. It is insoluble in chloroform, carbon tetra chloride, 0.1M Hydrochloric acid.

Dapoxetine is determined by dissolving in minimum quantity of distilled water and made further solution with distilled water. The solution was scanned between wavelength range of 200 and 400 nm in the UV region using distilled water as a blank. From the UV spectrum obtained, 281.5 nm and 295.5 nm was chosen as wavelength for further analysis of Dapoxetine. The Area under curve spectrum are shown in figure 3.

By using the selected wavelength, the absorbance of solution was observed and measured in different frequent intervals. It was found that the Dapoxetine having the stability for 4 hours and 30 minutes. So the same wavelength was selected for further analysis.

The different aliquots of Dapoxetine in distilled water prepared in the concentration range from 5-30 µg/mL. The absorbances of the same solution were measured at 281.5 nm and 295.5 nm. The linearity procedure was repeated for six times.

From the linearity analysis, the calibration curve was plotted by using concentration against absorbance. The optical characteristics of Dapoxetine are correlation coefficient, regression equation, slope, intercept, limit of detection, limit of quantification, sandell's sensitivity and standard error of mean were calculated. The reports are shown in the table 7. The average value of correlation coefficient from linearity studies was found to be 0.9996. It mentioned that the concentration of Dapoxetine has good linearity. The calibration graph are shown in figure 7.

The amount of Dapoxetine was determined in the test solution by using average values of slope and intercept. Repeated the same procedure for six times. The average percentage label claim of Dapoxetine present in tablet formulation (Prilyxet 30) was found to be 99.3 ± 0.08165. The percentage relative standard deviation value revealed that the proposed method has good precision. The results of this analysis are shown in table 8.

The precision of the proposed method was further ensured by carry out the recovery analysis. To the 50% of pre-analyzed tablet formulation, a known quantities of Dapoxetine standard solution was added at different levels (80%, 100% and 120% of quantification concentration). The absorbance of the recovery solutions were measured at 281.54 and 295.5 nm and the percentage purity of recovery analysis was found to be 100.74%.

It was revealed that the excipients did not interface in the tablet formulation of Dapoxetine. The recovery analysis are shown in table 9.

Further, the precision of the proposed method was confirmed by intraday and interday analysis of quantification studies, the quantification analysis was performed for three times in the same day and one time in the three successive days.

The %RSD values were found to be 0.057948 and 0.057948 for intraday and interday analysis of formulation, the low percentage RSD revealed that the precision of the proposed method was ensured. The results of interday and intraday analysis are shown in table 10.

The method was developed and validated by ruggedness analysis. It was performed by different days which may consists different analysts and different instruments. The % RSD values were found to be and 0.743303 for different instruments and different analysts respectively. The reports of ruggedness analysis were showed in table 11. Hence the precision was ensured by ruggedness analysis.

5.3 DERIVATIVE SPECTROPHOTOMETRIC METHOD

The various polar and non-polar solvents were carried out for Dapoxetine were Distilled water, methanol, ethanol, 0.1M Hydrochloric acid, acetone, ethyl acetate, isopropyl alcohol, acetonitrile, dimethyl formamide, diethyl ether, chloroform, carbon tetra chloride.

From the solubility studies, the Dapoxetine was found to be freely soluble in methanol and acetonitrile, ethyl acetate. Dapoxetine soluble in distilled water, acetone, and dimethyl formamide. It is insoluble in chloroform, carbon tetra chloride, 0.1M Hydrochloric acid.

Dapoxetine is determined by dissolving in minimum quantity of distilled water and made further solution with distilled water. The solution was scanned between wavelength range of 200 and 400 nm in the UV region using distilled water as a blank. The zero order spectrum obtained was derivatised to second order derivative spectrum. From the derivative spectra obtained, 241 nm and 234.5 nm were chosen as maxima and minima wavelength for further analysis of Dapoxetine. The derivative spectrum is shown in figure 4.

By using the selected wavelength, the absorbance of solution was observed and measured in different frequent intervals. It was found that the Dapoxetine having the stability for 4 hours and 30 minutes. So the same wavelength was selected for further analysis.

The different aliquots of Dapoxetine in distilled water were prepared in the concentration range from 5-30 µg/mL. The absorbance of the same solution were

measured at minima 234.5 nm and maxima 241 nm. The linearity procedure was repeated for six times. From the linearity analysis, the calibration curve was plotted by using concentration against absorbance. The optical characteristics of Dapoxetine are correlation coefficient, regression equation, slope, intercept, limit of detection, Limit of quantification, sandell's sensitivity and standard error of mean were calculated. The reports are shown in the table 12. The average value of correlation coefficient from linearity studies was found to be 0.9999. It mentioned that the concentration of Dapoxetine has good linearity. The calibration graph are shown in figure 8.

The amount of Dapoxetine was determined in the test solution by using average values of slope and intercept. Repeated the same procedure for six times. The average percentage label claim of Dapoxetine present in tablet formulation (prilyxet 30) was found to be 99.84 ± 0.869768. The percentage relative standard deviation value revealed that the proposed method has good precision. The results of those analysis are shown in table 13.

The precision of the proposed method was further ensured by carry out the recovery analysis. To the 50% of pre-analyzed tablet formulation, a known quantities of Dapoxetine standard solution was added at different levels (80%, 100% and 120% of quantification concentration). The absorbance of the recovery solutions were measured at 234.5 nm and 241 nm. The percentage purity of recovery analysis was found to be 100.12%.

It was revealed that the excipients did not interface in the tablet formulation of Dapoxetine. The recovery analysis are shown in table 14.

Further, the precision of the proposed method was confirmed by intraday and interday analysis of quantification studies, the quantification analysis was performed for three times in the same day and one time in the three successive days.

The %RSD values were found to be 0.692128 and 0.050103 for intraday and interday analysis of formulation, the low percentage RSD revealed that the precision of the proposed method was ensured. The results of interday and intraday analysis are shown in table 15.

The method was developed and validated by ruggedness analysis. It was performed by different days which may consists different analysts and different instruments. The % RSD values were found to be 0.071173 and 0.692128 for different instruments and different analysts respectively. The reports of ruggedness analysis were showed in table 16. Hence the precision was ensured by ruggedness analysis.

5.4 VISIBLE SPECTROPHOTOMETRIC METHOD

Dapoxetine has tertiary amino group, hence it was planned to treat with 0.01M Cerric Ammonium Sulphate and the resulting solution was tested for various color reactions with different chromogenic regents like MBTH, $Fecl_3$, PDAB, FC. Where MBTH reagent gave a stable purple colour with Dapoxetine in presence of oxidizing agent (0.01M Cerric Ammonium Sulphate). From solubility studies, the distilled water was selected as a choice of solvent for the proposed method.

The stability of the colored solution was found up to 2 hours and there is no change in absorbance. The 10 µg/mL solution was prepared and scanned from 400 to 800 nm. The visible spectrum was obtained, from the visible spectra shows a maximum absorbance at 437 nm. The visible spectrum shown in the figure 5.

Dapoxetine was found to yield a purple colored product with MBTH reagent and 0.01M Cerric Ammonium Sulphate and has absorbance maximum of 437 nm. Therefore studies were carried out to establish the mostly favorable condition for the formation of these colored products.

The influence of the concentration with constant volumes of the reagent on the reaction has been studied. Various concentration of MBTH reagent like 1mL, 2mL, 3mL, 4mL, and 5mL were tried by varying the parameter of 0.01M CAS concentration like 1mL, 2mL, 3mL, 4mL, and 5mL at a time, it was found that the optimum concentration of 0.01M Cerric Ammonium Sulphate was found to be 2 mL and that of MBTH reagent was found to be 2 mL.

Dapoxetine was dissolved in distilled water, and made up to the volume with distilled water to get a concentration of 1000 µg/mL. Then prepared 100 µg/mL solution using distilled water, from which a linearity of 5-30 µg/mL was made and measured the absorbance at 437nm. The calibration curve was made by using concentration against absorbance were shown in the figure 9.

The optical characteristics of Dapoxetine are correlation coefficient, regression equation, slope, intercept, Limit of detection, Limit of quantification, sandell's sensitivity and standard error of mean were calculated.

Correlation coefficient was found to be close to 1, intimate that it has good linearity and obey's Beer's Lambert's law. The limit of detection, limit of quantification were found from the linearity analysis by using average of slope and intercept values. The results are shown in Table 17.

Assay of commercial formulation involved using 15 µg/mL solution. The amount of drug present in the marked formulation was determined by average values of slope and intercept and the average percentage label claim was found to be 99.0 ± 0.250333. The results of this analysis are shown in table 18.

To determine the accuracy of the method by using recovery analysis, known amount of pure drug (80, 100 and 120% of quantification concentration) were added to the previously analyzed solutions. The absorbance of the recovery solutions were measured at 437 nm. The percentage recovery was found to 100.0%. The results of recovery analysis are shown in table 19.

The precision studies were carried out by intraday and interday repeatability studies for assay. The results of quantification for intraday and interday 0.300595 and 2.072575 respectively. The results are shown in table 20.

The method was developed and validated by ruggedness analysis. It was performed by different days which may consist different analysts and different instruments. The % RSD values were found to be 0.142132 and 0.070959 for different instruments and different analysts respectively. The reports of ruggedness analysis were showed in table 21. Hence the precision was ensured by ruggedness analysis

5.5 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD

Various mobile phase systems were prepared and trails were made with pre-coated plates to determine appropriate chromatographic conditions. The mobile phase finalized for HPTLC analysis was Acetonitrile : Ethyl acetate in the ratio of 9 : 1 v/v and the chromatogram was developed. The scanning of the developed plates shows a good peak shape.

The linearity of HPTLC method developed for Dapoxetine was evaluated by injecting the standard drug solutions of different concentration ranging from 1 to 6 µg/mL and the peak areas obtained are presented in figure 12-17. The calibration curve was made by using concentration against absorbance were shown in the figure 18.

The various optical parameters like Regression equation, Slope, Intercept, Sandell's sensitivity, standard error of mean were calculated and the best results are presented in Table 22

The Assay determined the contents of Dapoxetine in formulation (Priliyxet 30) were found to be 100.45%. The assay results presented in the table 23. The chromatograms are shown in figure 19.

The Accuracy and precision was determined for Dapoxetine by fortifying sample with standard drug substance at a concentration range 2.4, 3.0, 3.6 µg/mL and overall recovery determined, The data presented in the table 24. The Chromatograms are shown in figures 20 and respectively. The results of quantification for intraday and interday 0.55075 and 0.30550 respectively. The results are shown in table 25.

Hence it is concluded that the developed UV visible and HPTLC methods are found to be simple, precise and accurate for the analysis of Dapoxetine in pure form and in pharmaceutical formulation.

Among the established analytical methods, HPTLC method can be applied for regular analysis of Dapoxetine from pure and pharmaceutical dosage form.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Standard analytical studies for newer formulation drugs may not be available in pharmacopeias, since it is necessary to develop analytical estimation of which are simple, accurate, precise, economic, specific, rapid and linear.

Methods employed were

6.1 UV spectrophotometric method

6.2 Area under curve method

6.3 Derivative spectrophotometric method

6.4 Visible spectrophotometric method

6.5 HPTLC method.

6.1 UV SPECTROPHOTOMETRIC METHOD

The estimation of Dapoxetine was achieved by UV spectrophotometric method. By considering the solubility status and stability studies, the distilled water was selected as suitable solvent and further analysis were made by using the distilled water. 10 µg/mL solution of Dapoxetine was prepared from the stock solution and scanned in the UV region and λmax was found at 292nm. Dapoxetine obey's Beer's law and it was ensured from the calibration graph in the range of 5-30 µg/mL. From marketed formulation, 15 µg/mL concentration was prepared by using the same solvent. The percentage label claim of Dapoxetine in tablet formulation was found to be 100.6 ± 0.428657. The precision of the proposed method was carried out by performing the repeated analysis. The recovery studies were also performed to determine the accuracy of the UV method by adding the 50% of pre-analysed formulation. The average percentage recovery

analysis for formulation (Prilyxet 30) was found to be 100.3%. The precision of the same method was determined by intraday and interday analysis on assay. Also the % RSD values were found to be 0.210207 and 0.013981 for intraday and interday analysis of formulation. Also the precision analysis was further performed by ruggedness analysis using different analysts and different instruments.

6.2 AREA UNDER THE CURVE METHOD

The evaluation of Dapoxetine was reached by Area under curve method. From the analysis of solubility status and stability studies, the water was selected as suitable solvents for further analysis. 10 µg/mL solution of Dapoxetine was prepared from the stock solution and scanned in the UV region. From the area under curve spectrum, the area between 281.5 nm and 295.5 nm was selected as wavelength for further analysis. Dapoxetine was obey's beer's law and it was ensured from the calibration graph in the range of 5-30 µg/mL. From the commercial formulation, 15 µg/mL concentration of Dapoxetine was prepared by using the same solvent. The percentage label claim of Dapoxetine in tablet formulation was found to be 99.3 ± 0.08165. The precision of the proposed method was performed by carry out the repeated analysis. The recovery analysis was also performed to determine the accuracy of the area under curve method by adding the 50% of pre-analysed formulation. The average percentage recovery analysis for marketed formulation (Prilyxet 30) was found to be 100.74%. The precision of the some method was achieved by intraday and interday analysis on assay. Also the precision analysis was further confirmed by ruggedness analysis using different analysts and different instruments

6.3 DERIVATIVE SPECTROPHOTOMETRIC METHOD

The estimation of Dapoxetine was accomplished by derivative spectrophotometric method. In the view of solubility status and stability studies, the distilled water was picked out as best solvent for further analysis. 10 µg/mL of Dapoxetine solution was prepared from the stock solution and scanned in the UV region. From the derivative spectrum, 234.5 and 241 nm was selected as the minimum and maximum wavelength for further analysis. Dapoxetine obey's Beer's law and it was ensured from the calibration graph in the range 5-30 µg/mL. From the formulation, 15 µg/mL concentration was prepared by using the same solvent. The percentage label claim of Dapoxetine in the commercial tablet formulation was found to be 99.84 ± 0.869768. The precision of the proposed method was carried out by performing the repeated analysis. The recovery studies were also performed to determine the accuracy of the derivative spectrophotometric method by adding the 50% of pre-analysed formulation. The average percentage recovery analysis for formulation (Prilyxet 30) was found to be 100.12%. The precision of the same method was determined by intraday and interday analysis of formulation. Also the precision analysis was further performed by ruggedness analysis using different analysts and different instruments. The %RSD values were found to be 0.071173 for different instrument and 0.692128 for different analysts.

6.4 VISIBLE SPECTROPHOTOMETRIC METHOD

The assessment of Dapoxetine was studied by visible spectrophotometric method. The reagent used was MBTH reagent and 0.01M Cerrric Ammonium Sulphate 10 µg/mL solution of Dapoxetine was prepared from the stock solution and reagents were added to

get stable purple colour. The solution was scanned in the visible region (wavelength 400-800 nm). From the visible spectrum, 437 nm was selected as the maximum wavelength further analysis. Calibration graph was plotted by using the concentration versus absorbance. Dapoxetine obey's Beer's law and it was ensured from the calibration graph in the range of 5-30 µg/mL. From the tablet formulation, 15 µg/mL concentration was prepared by using the same solvent and reagents were added. The amount of Dapoxetine in tablet formulation was found to be 99.0 ± 0.25033. The precision of the planned method was carried out by performing the repeated analysis. The recovery analysis studies were also performed to determine the accuracy of the visible spectrophotometric method by adding the previously analyzed tablet formulation. The average percentage recovery analysis for formulation (Prilyxet 30) was found to be 100.00%. The precision of the same method was determined by intraday and interday analysis on assay. Also the precision analysis was further performed by ruggedness analysis using different analysts and different instruments.

6.5 HPTLC METHOD

Estimation Dapoxetine was achieved by HPTLC method, after performing various trails of mobile phase, Acetonitrile : Ethyl acetate (9 : 1 v/v) was selected as mobile phase. After eluting, the spots were scanned in the TLC chamber. From that, 292 nm was selected as analyzing wavelength. Calibration curve was plotted by concentration against peak area.

The percentage label claim of Dapoxetine in formulation (Prilyxet 30) was found to be 100.45 ± 0.29263. The precision of the method was studied by making repeated

analysis. The recovery studies also carried to ensure the accuracy of the method by adding concentration of drug to a pre-anlyzed formulation and the %RSD was found to be 0.2913.

The proposed methods could be successfully applied to estimate commercial pharmaceutical product containing Dapoxetine. Thus the above studies and finding will enable the above quantification of the drug for the investigation in the field of analytical chemistry.

Among the established analytical methods, HPTLC method was found to be more precise, time consuming and accurate.

FIGURES

FIGURE.1

IR SPECTRUM OF DAPOXETINE

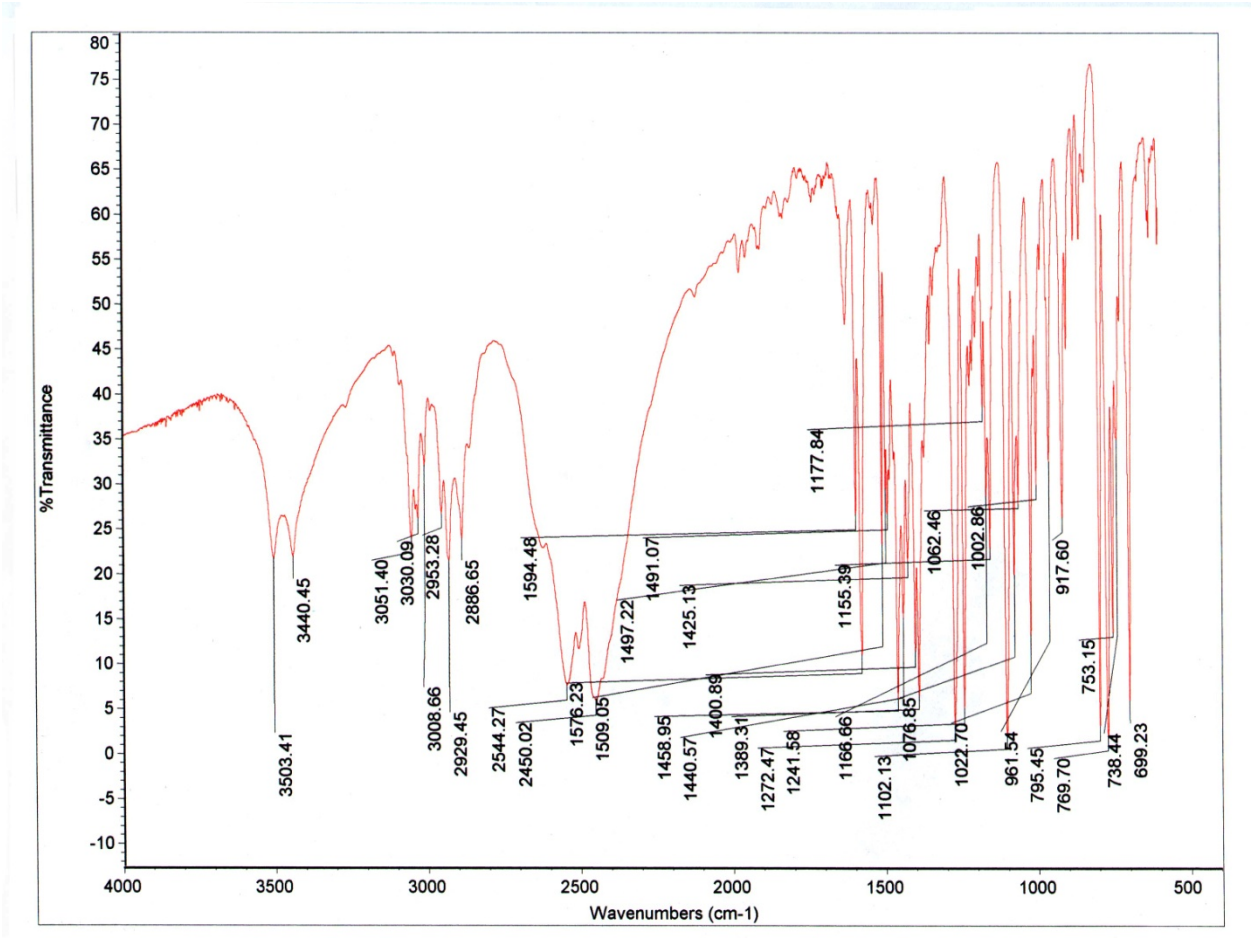


FIGURE.2

UV SPECTRUM OF DAPOXETINE IN

DISTILLED WATER

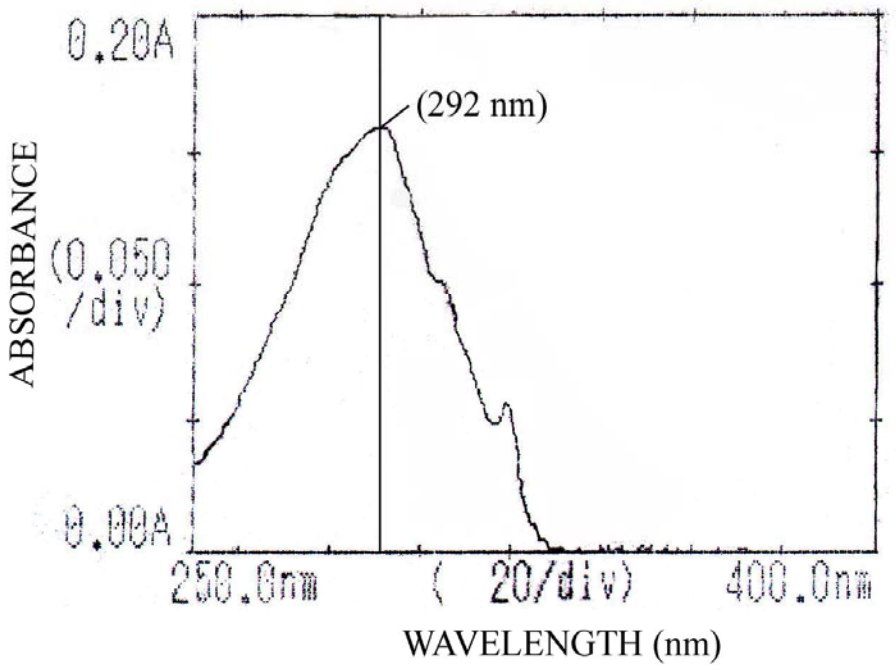


FIGURE.3

AREA UNDER CURVE SPECTRUM OF DAPOXETINE IN DISTILLED WATER

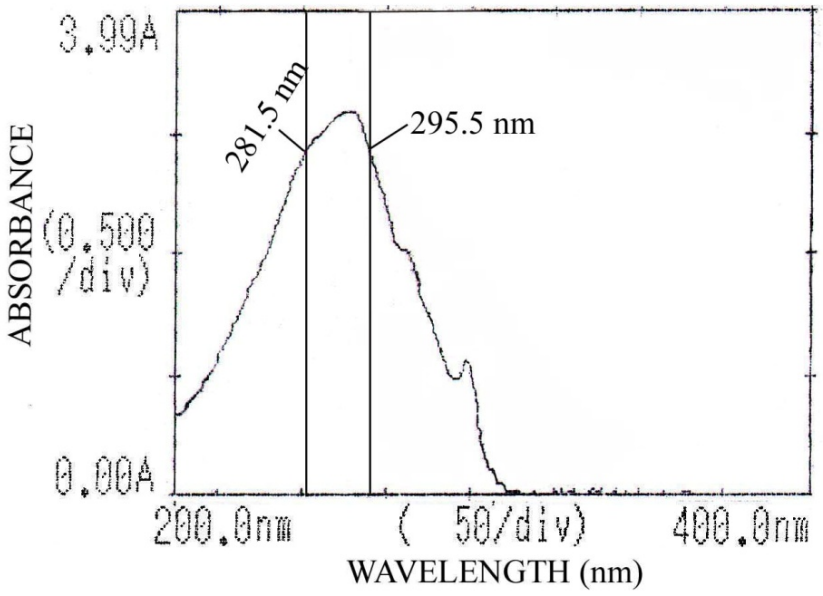


FIGURE.4

DERIVATIVE SPECTRUM OF DAPOXETINE IN DISTILLED WATER

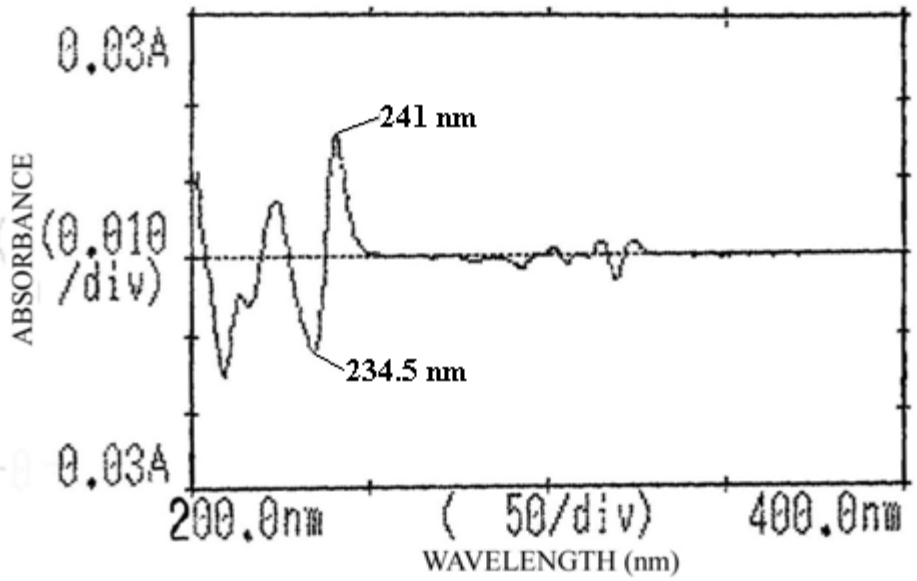


FIGURE.5

VISIBLE SPECTRUM OF DAPOXETINE IN DISTILLED WATER

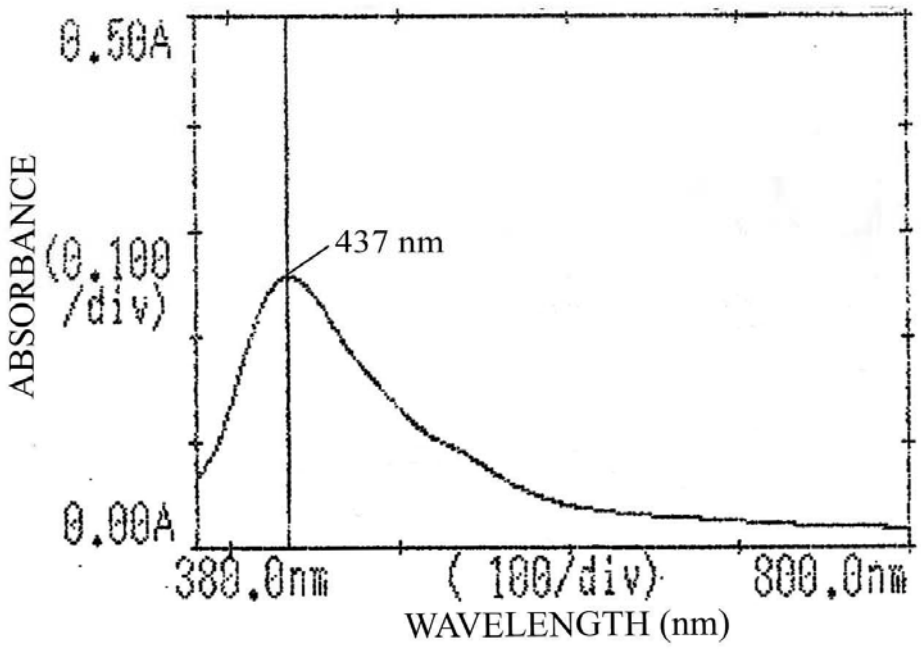


FIGURE.6

CALIBRATION CURVE OF DAPOXETINE BY UV SPECTROPHOTOMETRIC METHOD USING DISTILLED WATER AT 292 nm.

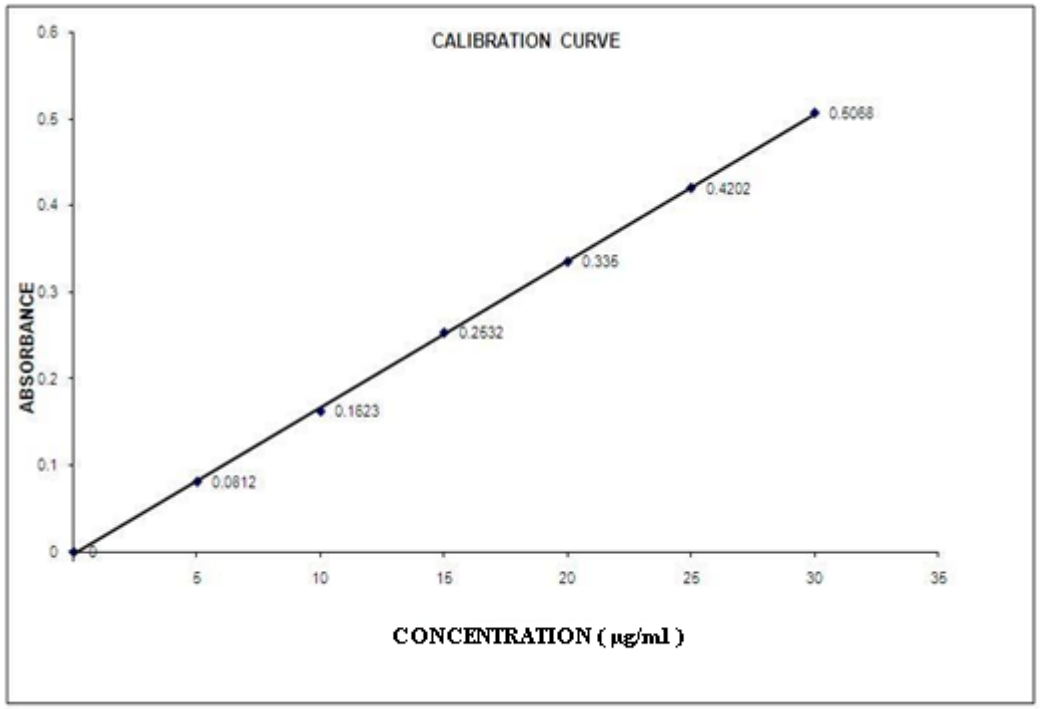


FIGURE.7

CALIBRATION CURVE OF DAPOXETINE BY AREA UNDER CURVE METHOD USING DISTILLED WATER AT 281.5 nm AND 295.5 nm.

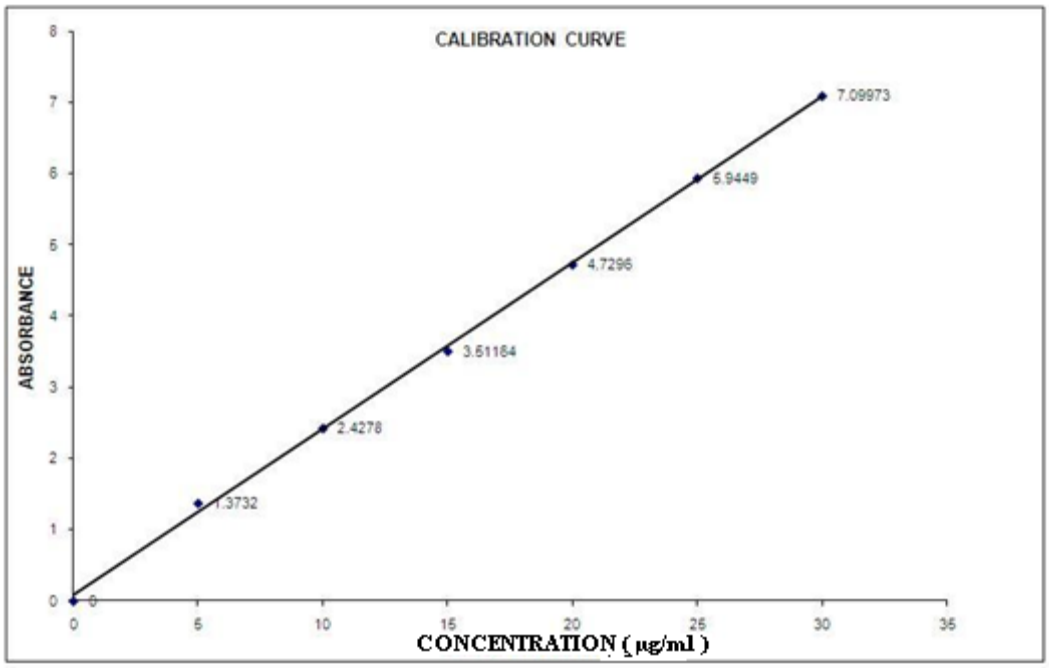


FIGURE.8

CALIBRATION CURVE OF DAPOXETINE BY DERIVATIVE SPECTROPHOTOMETRIC METHOD USING DISTILLED WATER AT 234.5 nm AND 241.0 nm.

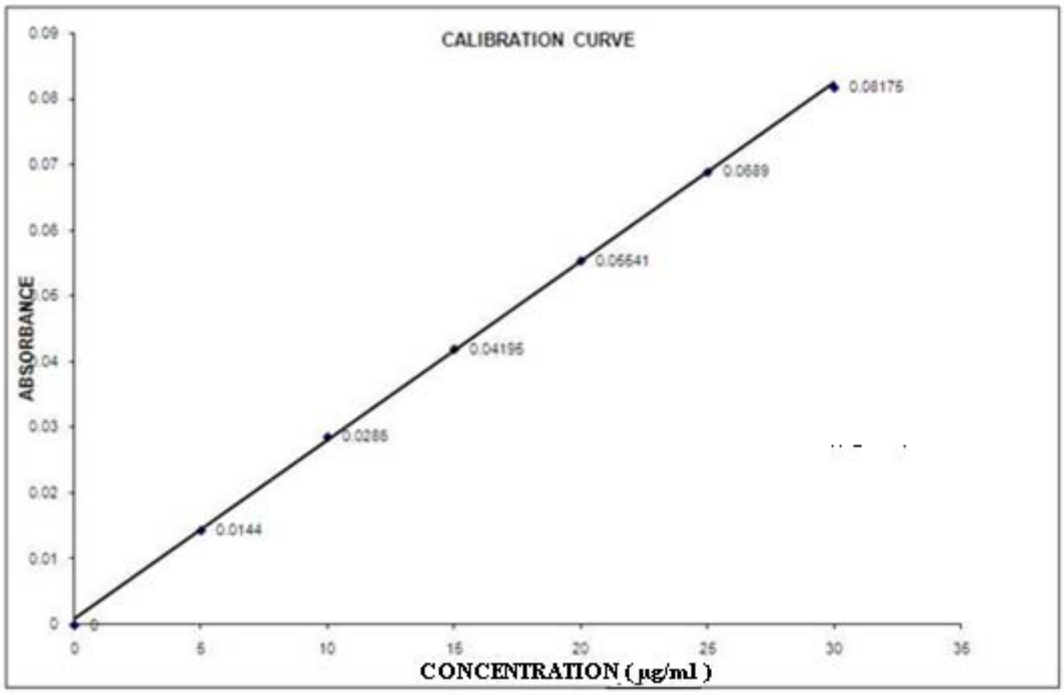


FIGURE.9

CALIBRATION CURVE OF DAPOXETINE BY VISIBLE SPECTROPHOTOMETRIC METHOD USING MBTH REAGENT AT 437nm.

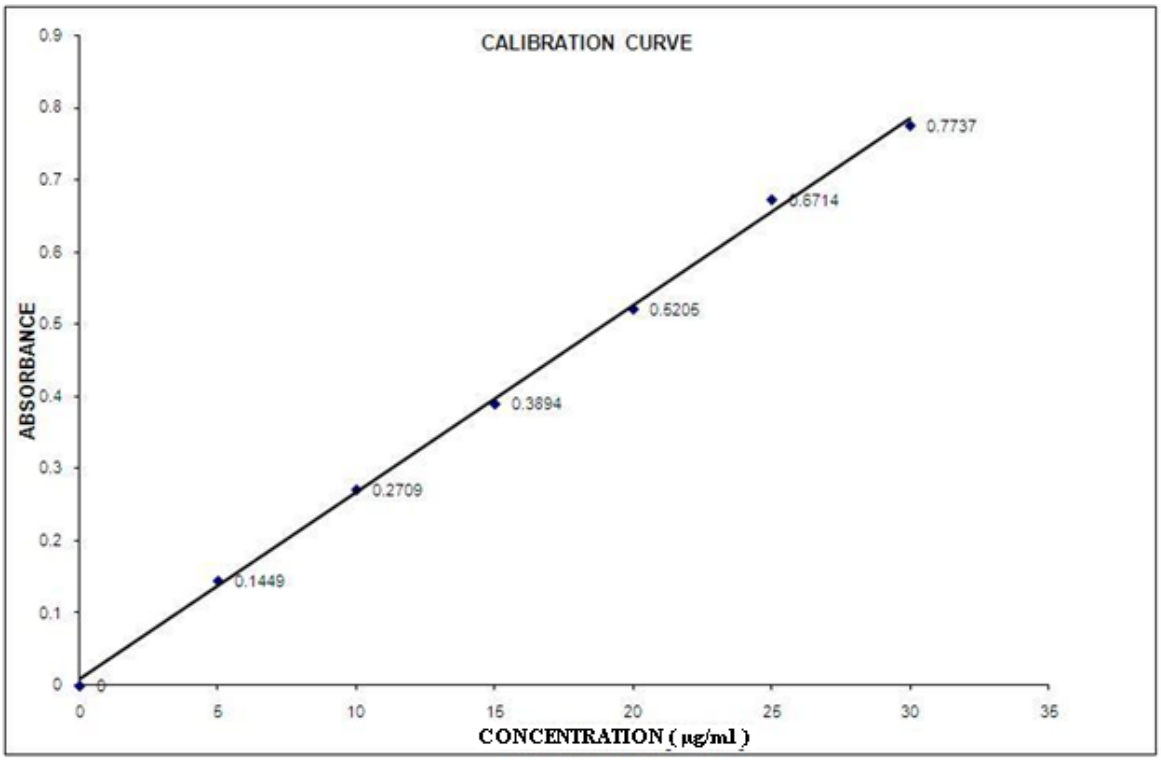


FIGURE.10

UV SPECTRA OF DAPOXETINE IN MOBILE PHASE

ACETONITRILE : ETHYL ACETATE (9 : 1 V/V)

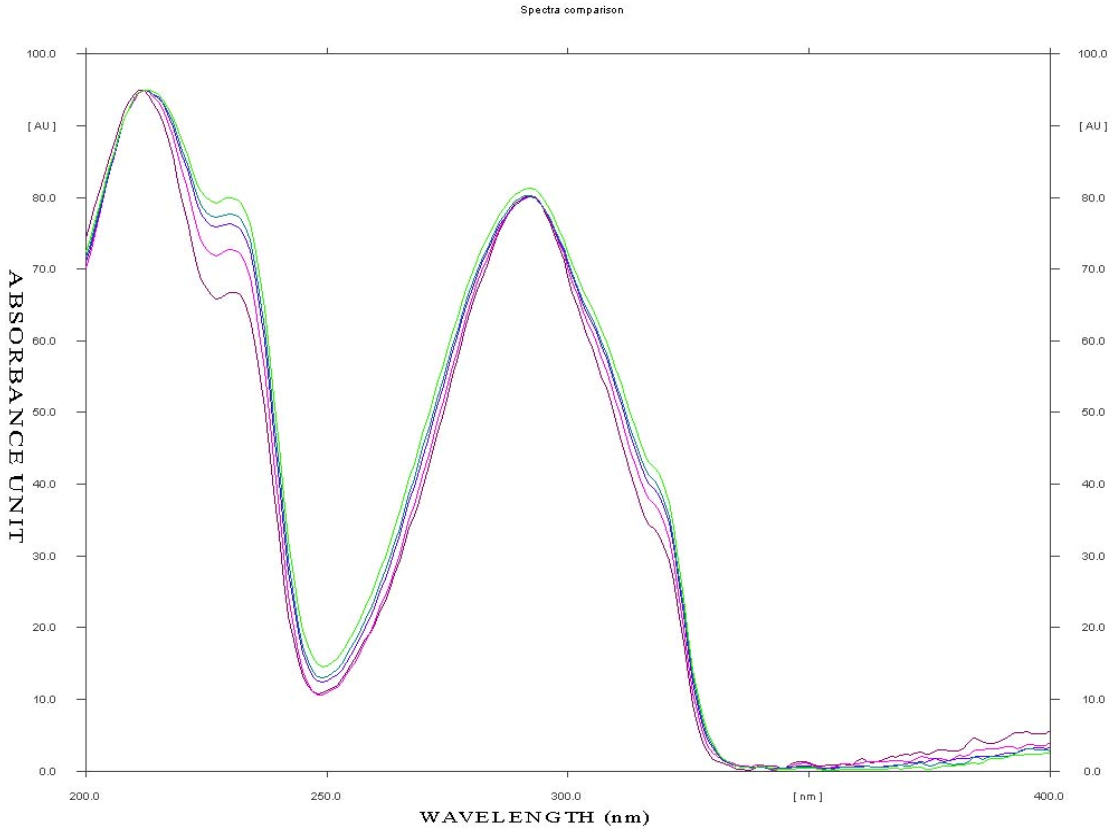


FIGURE.11

SPECTRAL CONFORMATION OF STANDARD DAPOXETINE WITH FORMULATION

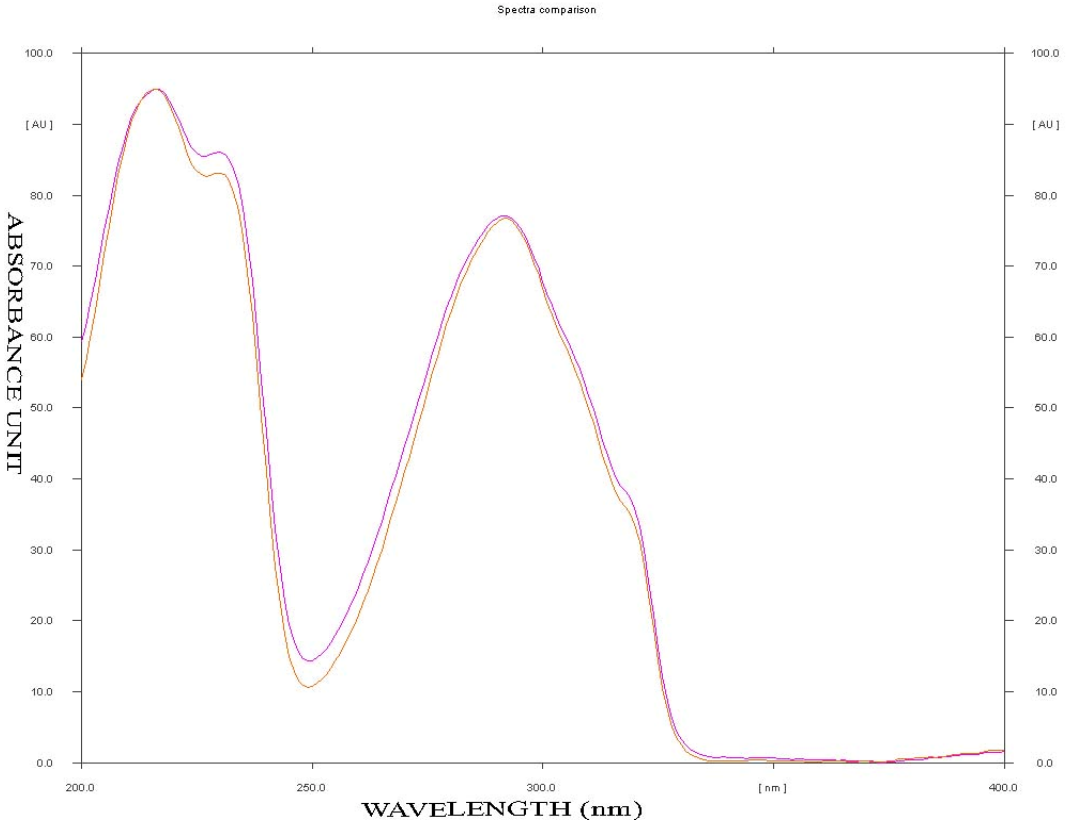


FIGURE.12

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTC METHOD

(1µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.54 Rf	7.0 AU	0.58 Rf	38.8 AU	100.00%	0.61 Rf	3.9 AU	1523.9	100.00%	Dapoxetine

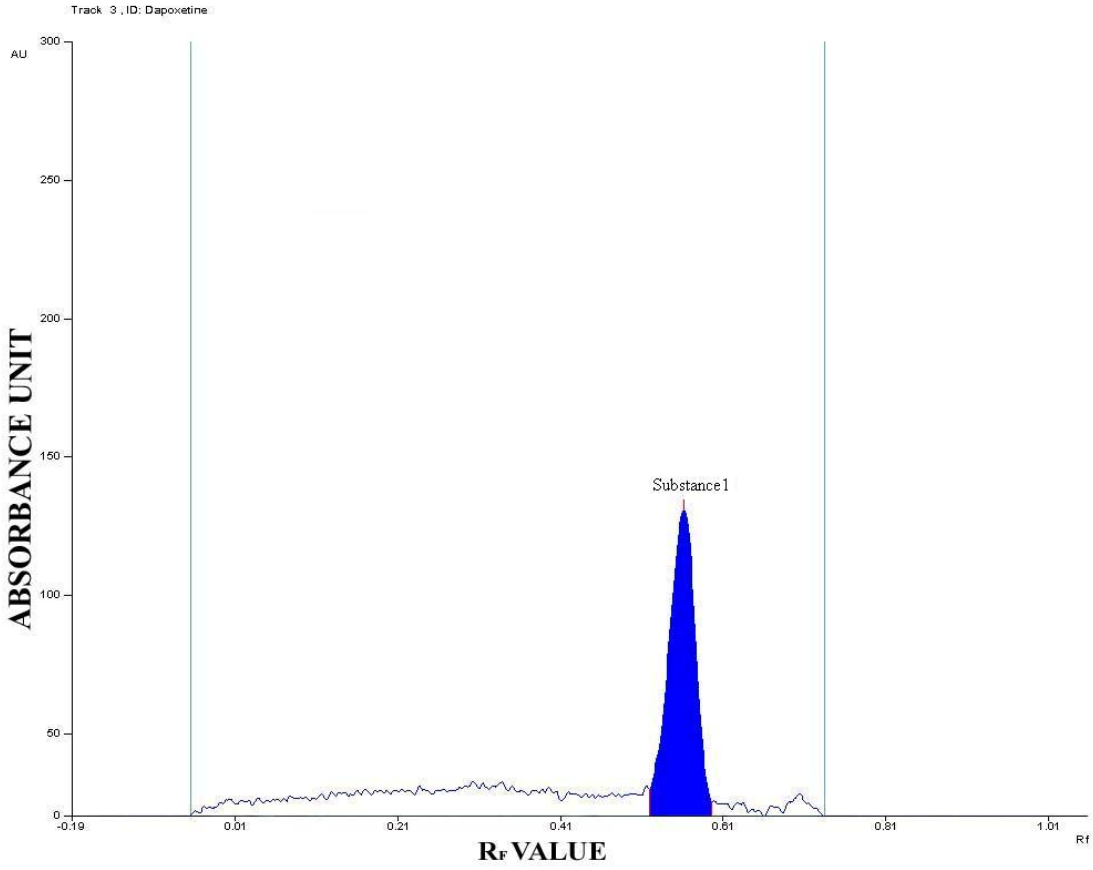


FIGURE.13

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD

(2µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
2	1	0.53 Rf	6.7 AU	0.58 Rf	76.9 AU	100.00%	0.63 Rf	2.1 AU	2859.8	100.00%	Dapoxetine

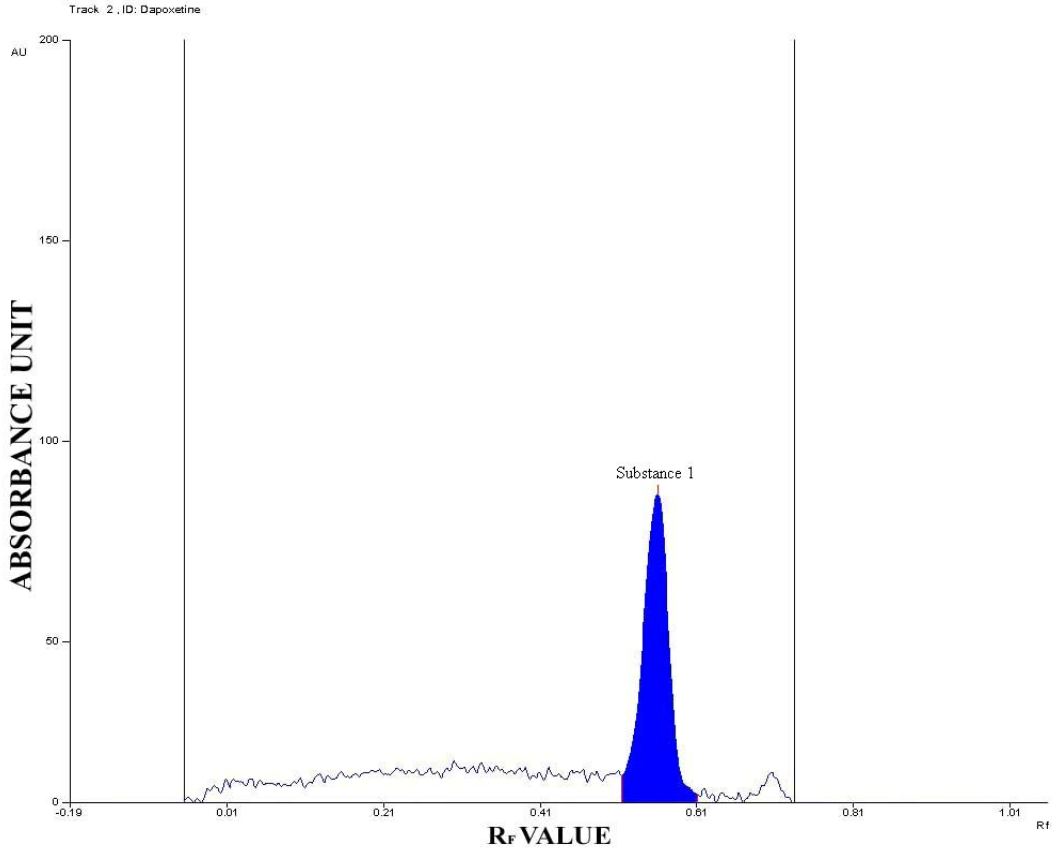


FIGURE.14

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD

(3 µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	1	0.54 Rf	9.8 AU	0.58 Rf	110.9 AU	100.00%	0.62 Rf	5.3 AU	4205.3	100.00%	Dapoxetine

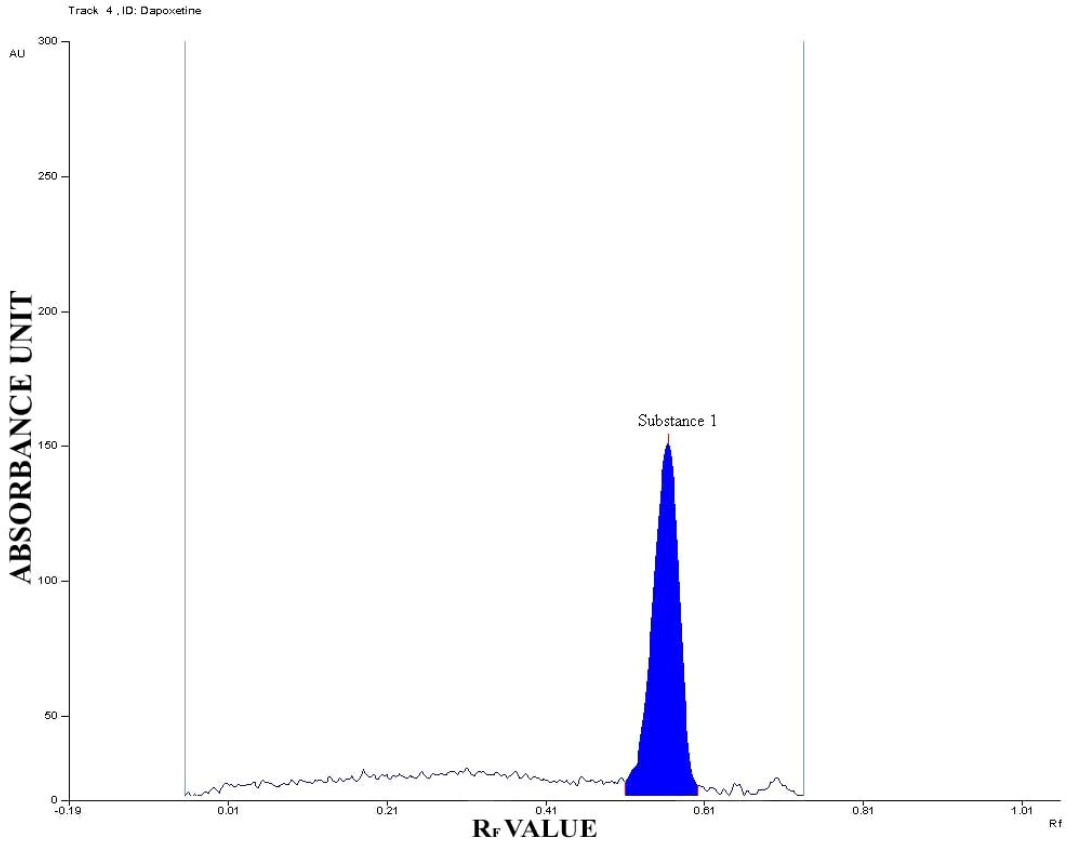


FIGURE.15

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD

(4 µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.53 Rf	5.4 AU	0.58 Rf	130.9 AU	100.00%	0.62 Rf	4.1 AU	5299.1	100.00%	Dapoxetine

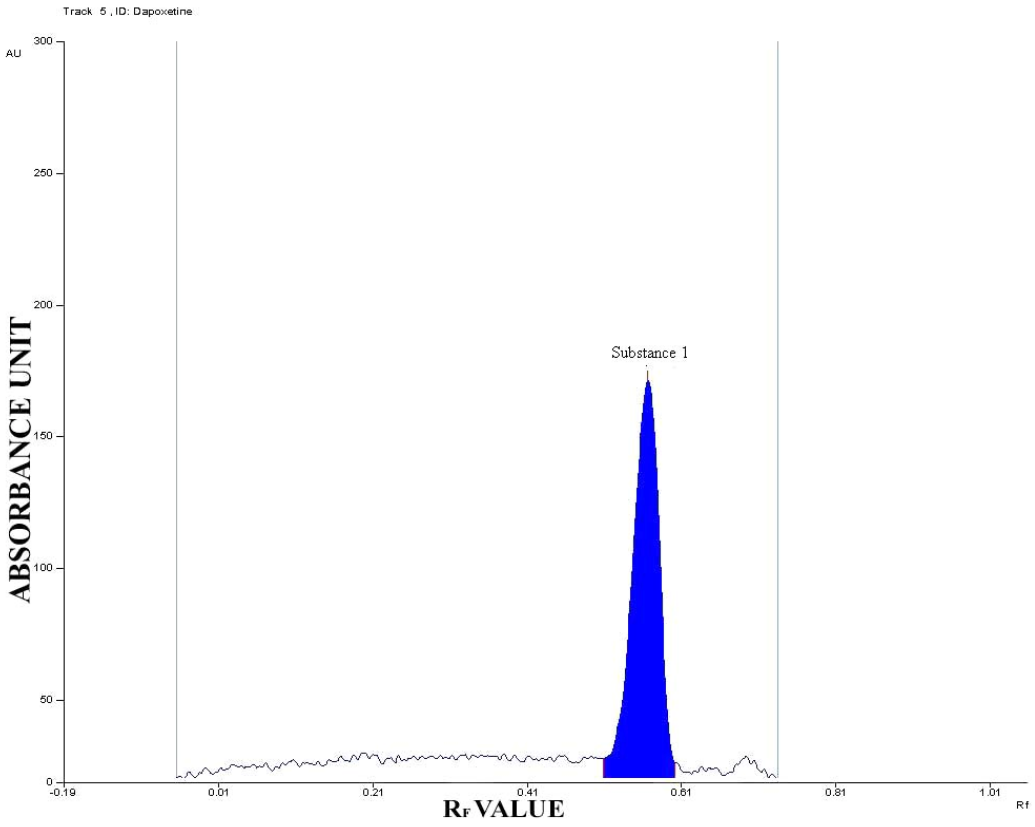


FIGURE.16

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD

(5 µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
5	1	0.53 Rf	7.4 AU	0.58 Rf	151.3 AU	100.00%	0.62 Rf	5.8 AU	6466.2	100.00%	Dapoxetine

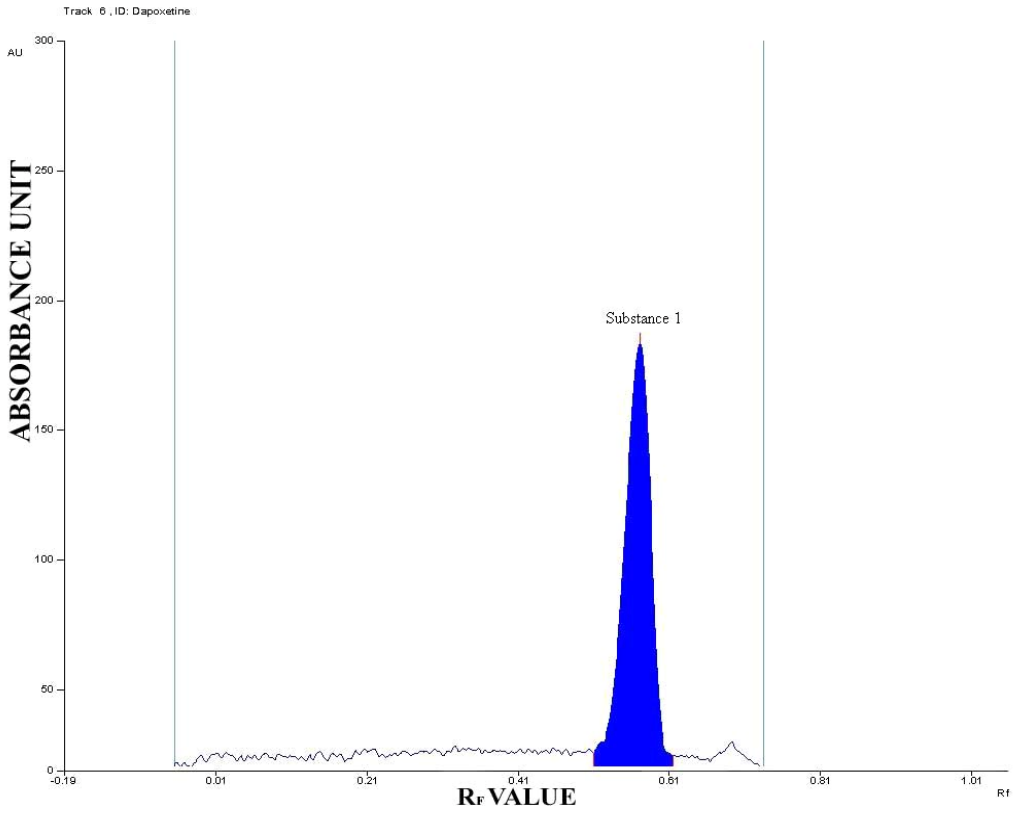


FIGURE.17

LINEARITY CHROMATOGRAM OF DAPOXETINE BY HPTLC METHOD

(6 µg/mL)

S.No	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
6	1	0.53 Rf	5.4 AU	0.59 Rf	163.7 AU	100.00%	0.63 Rf	4.4 AU	7638	100.00%	Dapoxetine

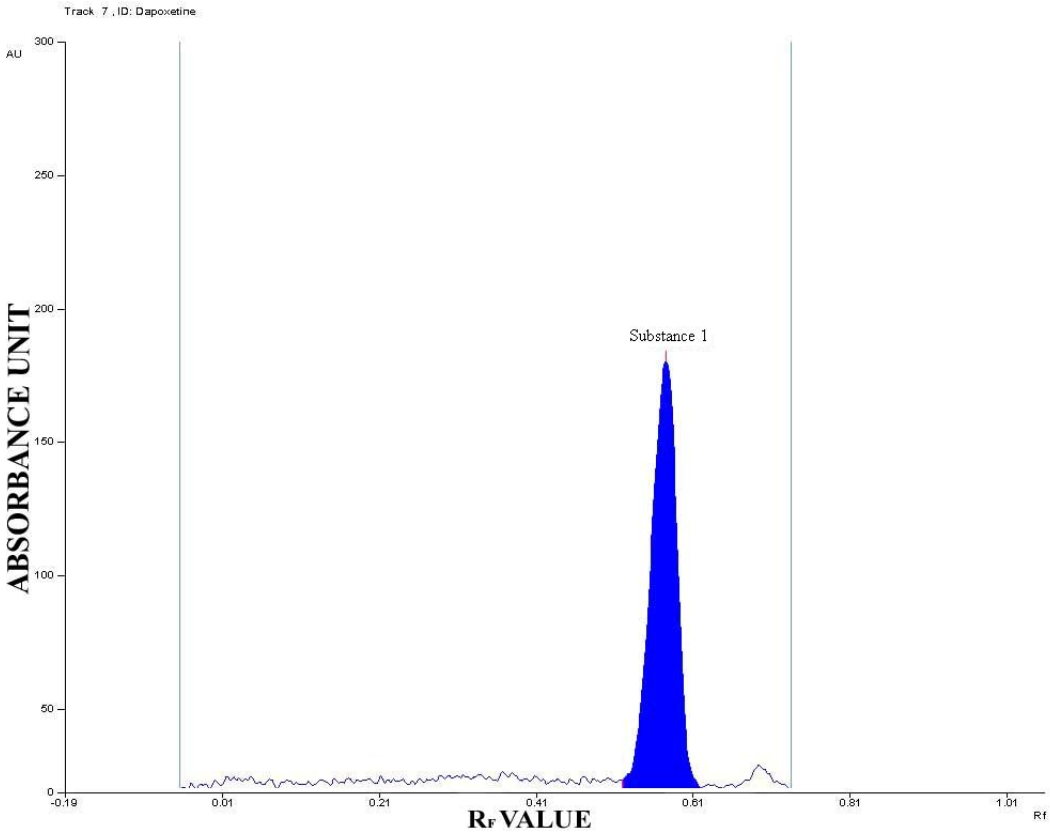


FIGURE.18

CALIBRATION CURVE OF DAPOXETINE BY HPTLC METHOD AT 292 nm

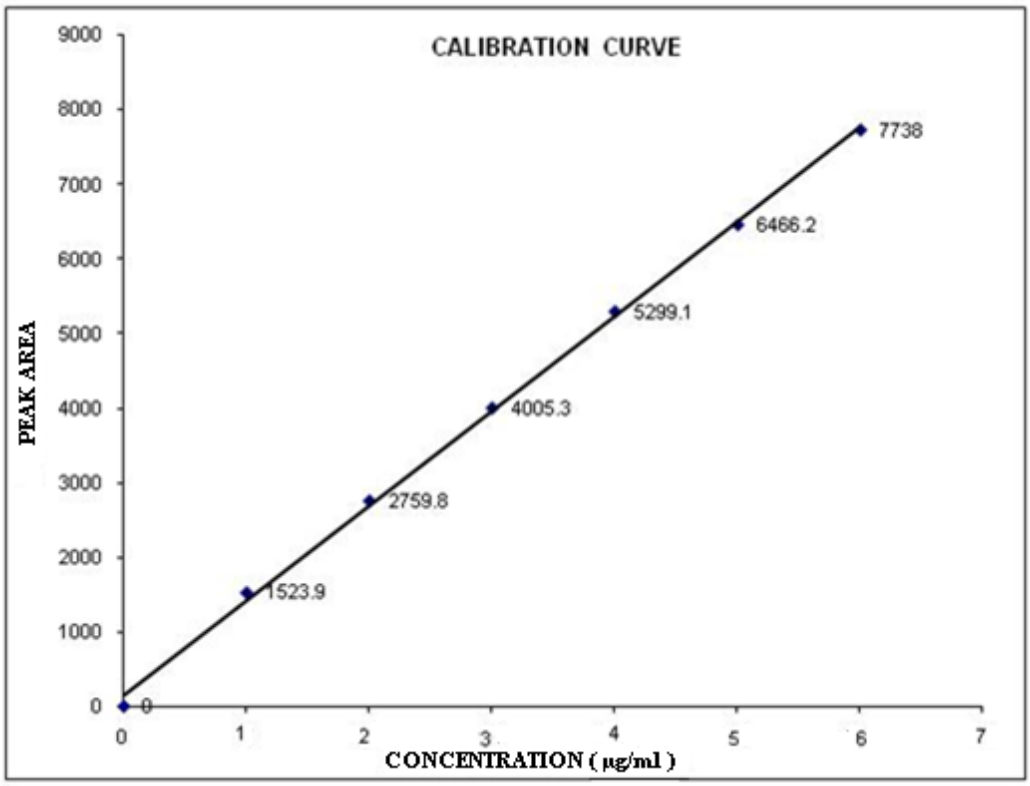


FIGURE.19

CHROMATOGRAM ANALYSIS OF FORMULATION – (PRILYXET 30)

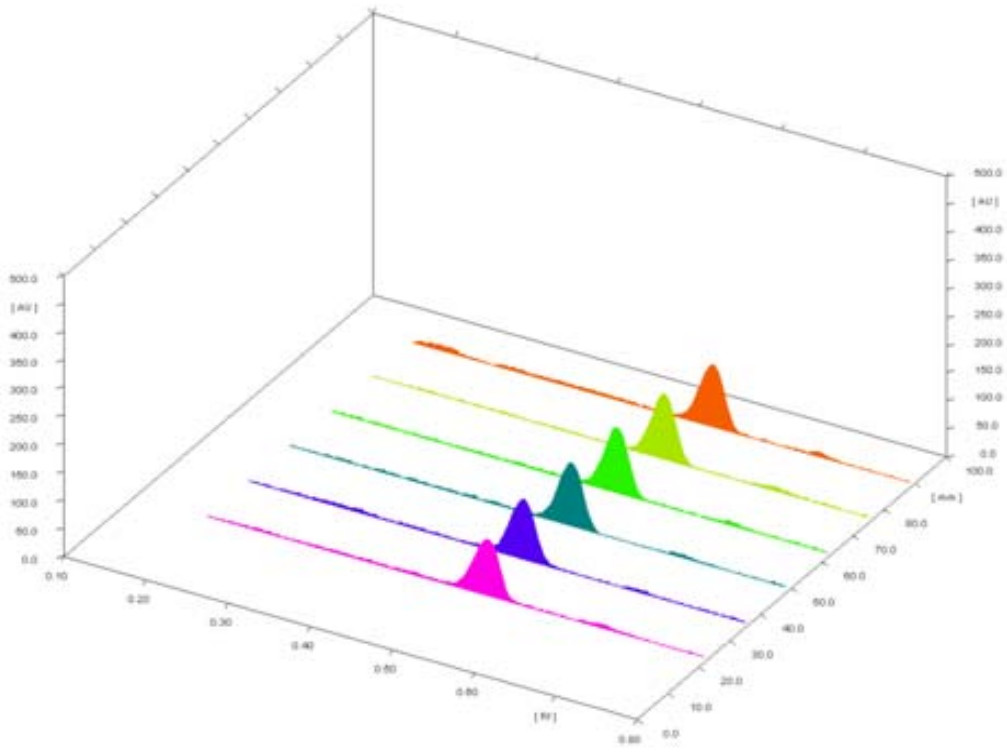
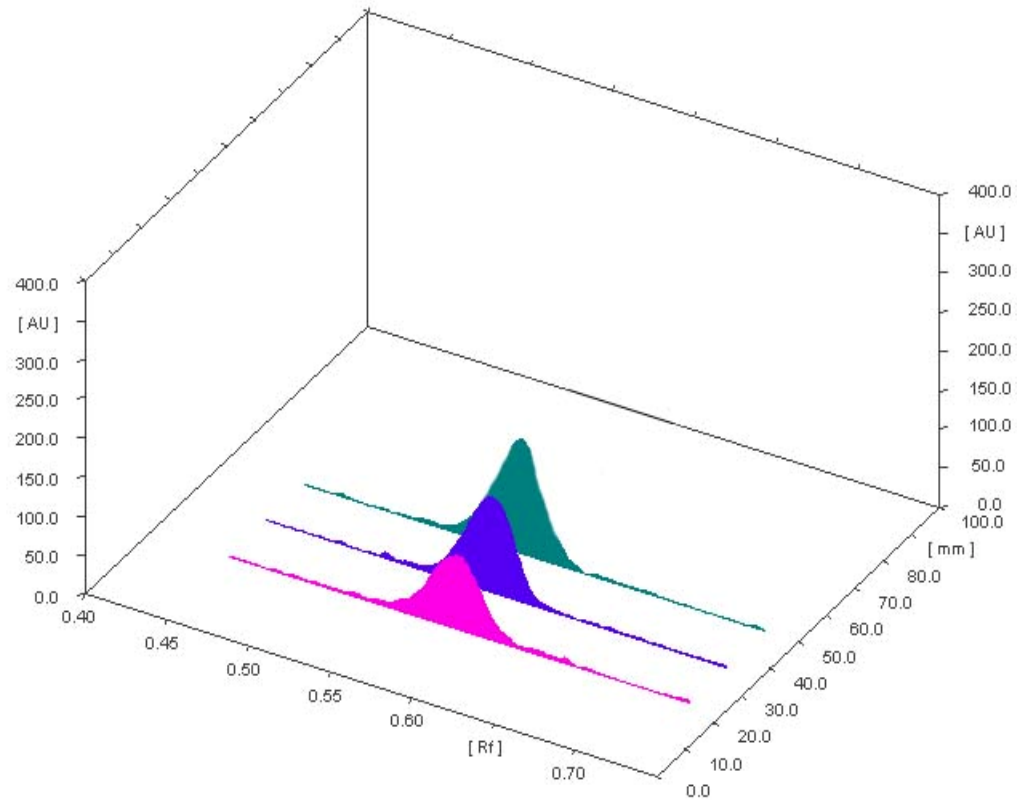


FIGURE.20

CHROMATOGRAM OF RECOVERY OF FORMULATION

(PRILYXET 30) – 2.4 µg/mL, 3.0 µg/mL, 3.6 µg/mL



TABLES

TABLE.1

SOLUBILITY STUDIES FOR DAPOXETINE HYDROCHLORIDE IN POLAR AND NON-POLAR SOLVENTS

S.No	Solvent	Solubility	Solubility status
1.	Water	1 in 200 µL	Soluble
2.	0.1 M Hydrochloric acid	More than 10,000 parts	Insoluble
3.	Ethanol	1 in 50 µL	Freely soluble
4.	Methanol	1 in 50 µL	Freely soluble
5.	Acetone	1 in 100 µL	Soluble
6.	Ethyl acetate	1 in 50 µL	Freely soluble
7.	Isopropyl alcohol	1 in 50 µL	Freely soluble
8.	Acetonitrile	1 in 50 µL	Freely soluble
9.	Dimethyl formamide	1 in 100 µL	Soluble
10.	Diethyl ether	More than 10,000 parts	Insoluble
11.	Chloroform	More than 10,000 parts	Insoluble
12.	Carbon tetra chloride	More than 10,000 parts	Insoluble

TABLE.2

OPTICAL CHARACTERISTICS OF DAPOXETINE HYDROCHLORIDE BY UV SPECTROPHOTOMETRIC METHOD

S.No	Parameters	UV spectrophotometric method
1.	λmax (nm)	292 nm
2.	Beer's law limit (µg/mL)	5-30
3.	Correlation coefficient (r)	0.9999
4.	Regression equation (Y=mx+c)	Y= 0.016936 x + - 0.00280
5.	Slope (m)	0.016936
6.	Intercept (c)	-0.00280
7.	LOD (µg/mL)	0.736215896
8.	LOQ (µg/mL)	2.23095726
9.	Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.059044
10.	Standard error of mean	0.002593.

TABLE.3

QUANTIFICATION OF FORMULATION (PRILYXET 30) BY UV SPECTROPHOTOMETRICMETHOD

S.No	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found	S.D (+/-)	%R.S.D	S.E
1.	30	29.99	99.66%	100.6%	0.428657	0.428386	0.011907
2.	30	30.17	100.5%				
3.	30	29.97	99.9%				
4.	30	30.21	100.7%				
5.	30	29.96	99.86%				
6.	30	29.93	99.76%				

TABLE.4

RECOVERY STUDIES OF FORMULATION (PRILYXET 30) BY UV SPECTROPHOTOMETRICMETHOD

S. No	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)	Amount recovered (µg/mL)	Percentage recovered (%)	Average percentage recovered ± S.D	% R.S.D	S.E
1.	15.02	12	26.93	11.91	99.27	100.03% ± 1.292014	1.2918	0.1435
2.	15.02	15	30.25	15.23	101.5			
3.	15.02	18	32.90	17.88	99.33			

TABLE.5

INTRADAY AND INTERDAY PRECISION ANALYSIS OF FORMULATION (PRILYXET 30) BY UV SPECTROPHOTOMETRIC METHOD

S.No	Labeled obtained (mg/tab)	Percentage obtained (%)		S.D (+/-)		% R.S.D	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	30	99.10	99.0	0.208407	0.125831	0.210207	0.013981
2.	30	99.37	99.25				
3.	30	98.96	99.10				

TABLE.6

RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY UV SPECTROPHOTOMETRICMETHOD

S.No	Condition	Average percentage obtained (%)	S.D (+/-)	%R.S.D	S.E
1.	Analyst 1	99.61%	0.741148	0.743303	0.08285
2.	Analyst 2	99.01%			
3.	Instrument 1	99.21%	1.537314	1.559669	0.170813
4.	Instrument 2	99.07%			

TABLE.7

OPTICAL CHARACTERISTICS OF DAPOXETINE HYDROCHLORIDE BY AREA UNDER CURVE METHOD

S.No	Parameters	Area under curve method
1.	Selected area (nm)	281.5 - 295.5 nm
2.	Beer's law limit (µg/mL)	5-30
3.	Correlation coefficient (r)	0.9996
4.	Regression equation (Y=mx+c)	Y= 0.2338885 x + 0.075698306.
5.	Slope (m)	0.2338885
6.	Intercept (c)	0.075698306
7.	LOD (µg/mL)	2.609704467
8.	LOQ (µg/mL)	7.908195355
9.	Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.004275542
10.	Standard error of mean	0.075698306

TABLE.8

QUANTIFICATION OF FORMULATION (PRILYXET 30) BY AREA UNDER CURVE METHOD

S.No	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found	S.D (+/-)	%R.S.D	S.E
1.	30	29.79	99.3%	99.3%	0.08165	0.082198	0.002268
2.	30	29.78	99.3%				
3.	30	29.79	99.3%				
4.	30	29.78	99.3%				
5.	30	29.79	99.3%				
6.	30	29.79	99.3%				

TABLE.9

RECOVERY STUDIES OF FORMULATION (PRILYXET 30) BY AREA UNDER CURVE METHOD

S. No	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)	Amount recovered (µg/mL)	Percentage recovered (%)	Average percentage recovered ± S.D	% R.S.D	S.E
1.	14.90	12	26.94	12.04	100.33	100.74% ± 0.500633	0.496939	0.055626
2.	14.90	15	29.99	15.09	100.6			
3.	14.90	18	33.15	18.25	101.3			

TABLE.10

INTRADAY AND INTERDAY PRECISION ANALYSIS OF FORMULATION (PRILYXET 30) BY AREA UNDER CURVE METHOD

S.No	Labeled obtained (mg/tab)	Percentage obtained (%)		S.D (+/-)		% R.S.D	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	30	99.6	99.6	0.057735	0.057735	0.057948	0.057948
2.	30	99.7	99.7				
3.	30	99.6	99.6				

TABLE.11

RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY AREA UNDER CURVE METHOD

S.No	Condition	Average percentage obtained (%)	S.D (+/-)	%R.S.D	S.E
1.	Analyst 1	99.6	0.070711	0.070959	0.017678
2.	Analyst 2	99.7			
3.	Instrument 1	99.6	0.141421	0.142132	0.035355
4.	Instrument 2	99.6			

TABLE.12

OPTICAL CHARACTERISTICS OF DAPOXETINE HYDROCHLORIDE BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

S.No	Parameters	Derivative spectrophotometric method
1.	λmax (nm)	234.5 and 241 nm
2.	Beer's law limit (µg/mL)	5-30
3.	Correlation coefficient (r)	0.99984
4.	Regression equation (Y=mx+c)	Y= 0.002721857 x + 0.000745.
5.	Slope (m)	0.002721857
6.	Intercept (c)	0.000745
7.	LOD (µg/mL)	2.212485893
8.	LOQ (µg/mL)	6.704502708
9.	Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.367396211
10.	Standard error of mean	0.000564186

TABLE.13

QUANTIFICATION OF FORMULATION (PRILYXET 30) BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

S.No	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found	S.D (+/-)	%R.S.D	S.E
1.	30	30.07	100.2%	99.84%	0.869768	0.871089	0.02416
2.	30	30.23	100.7%				
3.	30	29.93	99.76%				
4.	30	29.55	98.5%				
5.	30	29.77	99.23%				
6.	30	30.23	100.7%				

TABLE.14

RECOVERY STUDIES OF FORMULATION (PRILYXET 30) BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

S. No	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)	Amount recovered (µg/mL)	Percentage recovered (%)	Average percentage recovered ± S.D	% R.S.D	S.E
1.	15.04	12	27.06	12.02	100.1	100.12% ± 0.4703	0.4697	0.5522
2.	15.04	15	29.99	14.95	99.66			
3.	15.04	18	33.15	18.11	100.6			

TABLE.15

INTRADAY AND INTERDAY PRECISION ANALYSIS OF FORMULATION (PRILYXET 30) BY DERIVATIVE SPECTROPHOTOMETRICMETHOD

S.No	Labeled obtained (mg/tab)	Percentage obtained (%)		S.D (+/-)		% R.S.D	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	30	100.5	99.6	0.69282	0.450925	0.692128	0.050103
2.	30	100.5	100.1				
3.	30	99.3	100.5				

TABLE.16

RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY DERIVATIVE SPECTROPHOTOMETRICMETHOD

S.No	Condition	Average percentage obtained (%)	S.D (+/-)	%R.S.D	S.E
1.	Analyst 1	99.03%	1.039447	0.692128	0.259862
2.	Analyst 2	100.5%			
3.	Instrument 1	99.3%	0.070711	0.071173	0.017678
4.	Instrument 2	99.3%			

TABLE.17

OPTICAL CHARACTERISTICS OF DAPOXETINE HYDROCHLORIDE BY VISIBLE SPECTROPHOTOMETRIC METHOD

S.No	Parameters	Visible Spectrophotometric method
1.	λmax (nm)	437
2.	Beer's law limit (µg/mL)	5-30
3.	Correlation coefficient (r)	0.9996
4.	Regression equation (Y=mx+c)	Y= 0.025741 x + 0.008289
5.	Slope (m)	0.025741
6.	Intercept (c)	0.008289
7.	LOD (µg/mL)	1.117265968
8.	LOQ (µg/mL)	3.385654449
9.	Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.039070785
10.	Standard error of mean	0.001399969

TABLE.18

QUANTIFICATION OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRICMETHOD

S.No	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found	S.D (+/-)	%R.S.D	S.E
1.	30	29.67	98.9%	99.0%	0.250333	0.252692	0.006954
2.	30	29.77	99.2%				
3.	30	29.71	99.0%				
4.	30	29.61	98.7%				
5.	30	29.77	99.2%				
6.	30	29.83	99.4%				

TABLE.19

RECOVERY STUDIES OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRICMETHOD

S. No	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)	Amount recovered (µg/mL)	Percentage recovered (%)	Average percentage recovered ± S.D	% R.S.D	S.E
1.	14.92	12	27.01	12.09	100.0	100.00% ± 0.057716	0.0577	0.0064
2.	14.92	15	30.05	15.13	100.10			
3.	14.92	18	33.01	18.07	100.0			

TABLE.20

INTRADAY AND INTERDAY PRECISION ANALYSIS OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRICMETHOD

S.No	Labeled obtained (mg/tab)	Percentage obtained (%)		S.D (+/-)		% R.S.D	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	30	101.7	101.7	0.305505	2.080865	0.300595	2.072575
2.	30	101.9	98.0				
3.	30	101.3	101.5				

TABLE.21

RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY VISIBLE SPECTROPHOTOMETRICMETHOD

S.No	Condition	Average percentage obtained (%)	S.D (+/-)	%R.S.D	S.E
1.	Analyst 1	101.12	0.091924	0.090847	0.022981
2.	Analyst 2	101.25			
3.	Instrument 1	101.9	2.12132	2.112869	0.53033
4.	Instrument 2	98.9			

TABLE.22

OPTICAL CHARACTERISTICS OF DAPOXETINE BY HPTLC METHOD

S.No	Parameters	HPTLC method
1.	λmax (nm)	292 nm
2.	Beer's law limit (µg/mL)	5-30
3.	Correlation coefficient (r)	0.9995
4.	Regression equation (Y=mx+c)	Y= 1288.04881 x + 131.6392
5.	Slope (m)	1288.04881
6.	Intercept (c)	131.6392
7.	Sandell's sensitivity (µg/cm^2/0.001 A.U.)	7.76461
8.	Standard error of mean	4.18044

TABLE.23

QUANTIFICATION OF FORMULATION (PRILYXET 30) BY HPTLC METHOD

S. No	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found	S.D (+/-)	%R.S.D	S.E
1.	30	30.11	100.38				
2.	30	30.21	100.78	100.45	0.2926	0.2913	0.0325
3.	30	30.06	100.21				

TABLE.24

RECOVERY STUDIES OF FORMULATION (PRILYXET 30) BY HPTLC METHOD

S. No	Amount present (µg/ML)	Amount added (µg/ML)	Amount estimated (µg/ML)	Amount recovered (µg/ML)	Percentage recovered (%)	Average percentage recovered ± S.D	% R.S.D	S.E
1.	3.06	2.4	5.45	2.39	99.59	99.69% ± 0.591805	0.5936	0.0657
2.	3.06	3.0	6.07	3.01	100.33			
3.	3.06	3.6	6.63	3.57	99.16			

TABLE.25

RUGGEDNESS ANALYSIS OF FORMULATION (PRILYXET 30) BY HPTLC METHOD

S.No	Labeled obtained (mg/tab)	Percentage obtained (%)		S.D (+/-)		% R.S.D	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	30	100.2	100.1	0.55075	0.30550	0.54711	0.304389
2.	30	100.7	100.7				
3.	30	100.1	100.3				

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